

1 Supplementary Notes

3 Instructions to use Matlab Code:

- 4 1. Copy program files (*.m and *.fig) into a separate folder and add the path to the Matlab search paths using:
5 *pathtool -> Add Folder -> Save*
6
- 7 2. Copy files containing superresolution images (*.csv) into a separate folder. Example files are included for random and
8 clustered simulated data. Molecules were assumed to be observed seven times on average (exponentially
9 distributed). Clusters were simulated at $3 \mu\text{m}^2$ and 75 nm radius. See Methods for more details.
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- 11 3. Execute *clustermask_createset.m*

12 Note: The GUI is optimized for Matlab release 2015b.

- 14 4. Press *Open Folder* to select the folder that contains the example files for analysis.

- 16 5. Adjust the following parameters if needed (or use default values):

18 ROI size (pixel)	size of recorded image
19 Pixelsize (in nm)	camera pixel size
20 Gridsize (pixel)	number of pixels used for binary mask
22 resulting pixelsize	here the effective pixel size of binary masks is automatically calculated from the given parameters (ROI size, Pixelsize, Gridsize). It is recommended to keep this value significantly below the localization precision.
26 Sig for gaussians (nm)	standard deviation of Gaussians used to represent localizations
27 Cut gauss at x times sig	radius at which Gaussians are set to zero
29 use same cellroi for all	check box to use the same ROI for all files
31 Figure parameters	check boxes to specify output figure parameters
33 TH1-6	defines thresholds; set to 0 to skip
35 Settings rho vs eta plot	check boxes to specify output plots; 36 edit parameters for reference curve

- 38 6. Hit *Process Files* to start the analysis. First a dialog window will open to specify ROIs for analysis. When ROIs for all
39 files have been drawn, the program will start to analyze the files in the specified folder. The user can also mark the
40 checkbox "*use same cellroi for all*", in order to reuse the same ROI for all files in the chosen folder.
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- 42 7. Results are saved in *result.m*, which contains the following output variables:

44 num_locs	array containing the calculated number of localizations outside ($\#_{\text{out}}$) and inside ($\#_{\text{in}}$) of apparent 45 clusters. Different thresholds are grouped pair-wise ($\#_{\text{out}}$, $\#_{\text{in}}$) in the order specified in 46 no_locs_legend
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- 1 num_locs_legend specifies the order of the columns in the variable num_locs
- 2 clust_area calculated clustered area (A_{in})
- 3 cell_area area of the ROI drawn in the dialog window (A)
- 4 files file names of analyzed files
- 5 eta array containing the values for relative clustered area (rows correspond to
- 6 selected files; columns correspond to selected thresholds)
- 7 rho array containing values for localization density inside clusters (rows correspond
- 8 to selected files; columns correspond to selected thresholds)
- 9 fit struct containing the fitted parameters for all thresholds
- 10
- 11 8. Output graphs show the normalized ρ/η plot including the reference curve for randomly distributed data (red). Data
- 12 are plotted as blue circles. Results from example files will be comparable to **Figure 1**.