Installation

If you are going to use ClusterViSu as a plugin for SharpViSu, skip this chapter. The plugin is integrated in SharpViSu starting from version 1.2

Stand-alone application. Download and run “ClusterViSu_web_installer.exe” and follow instructions. If not already present, the required version of the MATLAB Compiler Runtime will be downloaded from the web and installed automatically.

You may also want to use a *portable stand-alone version*. Unpack archive “ClusterViSu.zip” to a preferred location. To run it, you will need to have MATLAB R2014a Compiler Runtime (v. 8.3) installed on your computed. It can be downloaded for free form the website of Matlab: http://fr.mathworks.com/products/compiler/mcr/

Matlab code. Add folder “ClusterViSu” to the search directory of Matlab. Note that the code was tested and compiled in Matlab R2014a.

Running ClusterViSu

Stand-alone application. Run “ClusterViSu.exe” (default path: “%ProgramFiles%\ClusterViSu\application\ClusterViSu.exe”, could have been changed during installation)

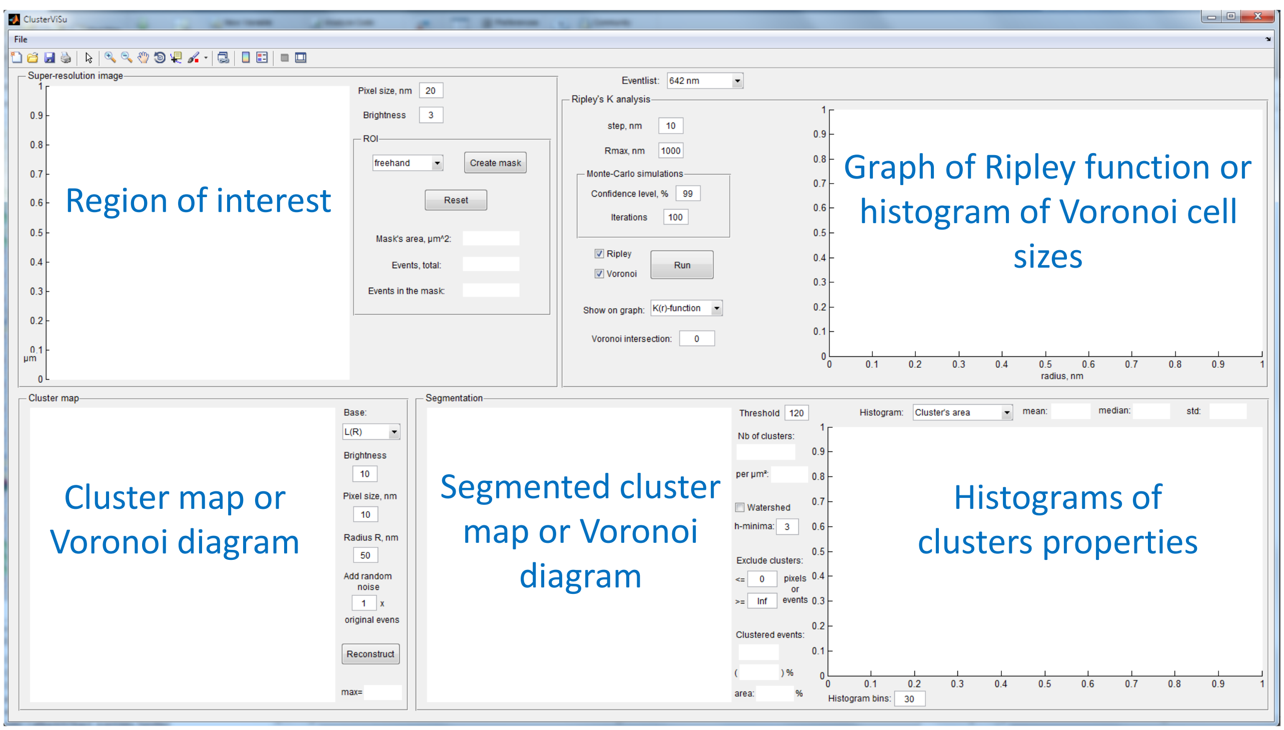
Matlab code. Type in the command line:

ClusterViSu

It will run the function ClusterViSu.m

SharpViSu plugin. Go to Plugins - ClusterViSu

The main window of the program will appear



Loading localization tables

Software can store in memory data for one or two channels. First, select the channel ("red" or "green", named for convenience) of your data in the popup menu "Eventlist".

Go to File – open eventlist and choose the format of your data. The following formats are supported:

(x, y) ascii table of localization coordinates;
Leica LAS AF (ascii, both 2D and 3D versions supported);
QuickPALM;
RapidSTORM;
Micro-Manager Localization Microscopy plugin (text files);
SharpViSu (internal format used in the software).

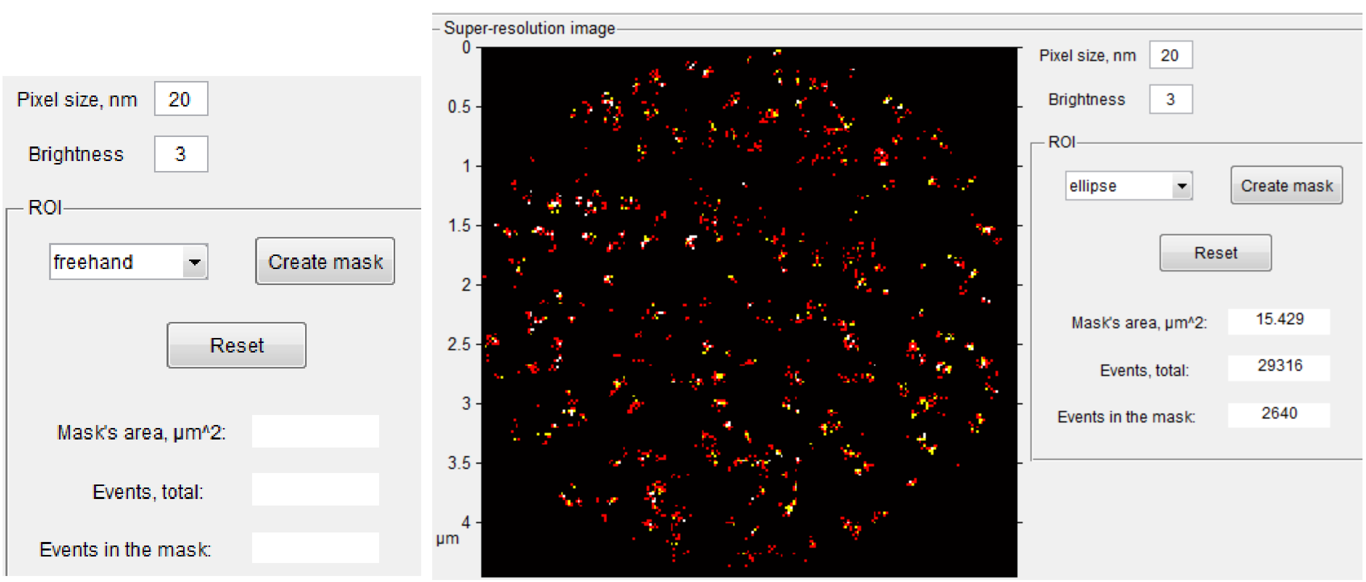
Press "Browse…" and choose a localization table. Please note that for Micro-Manager LM data all the commas will be replaced by dots in the original file.

If you run ClusterViSu as a plugin from SharpViSu, the procecced data will be authomatically transferred from SharpViSu to ClusterViSu, preserving the channel assignment.

Selection of a region of interest (ROI)

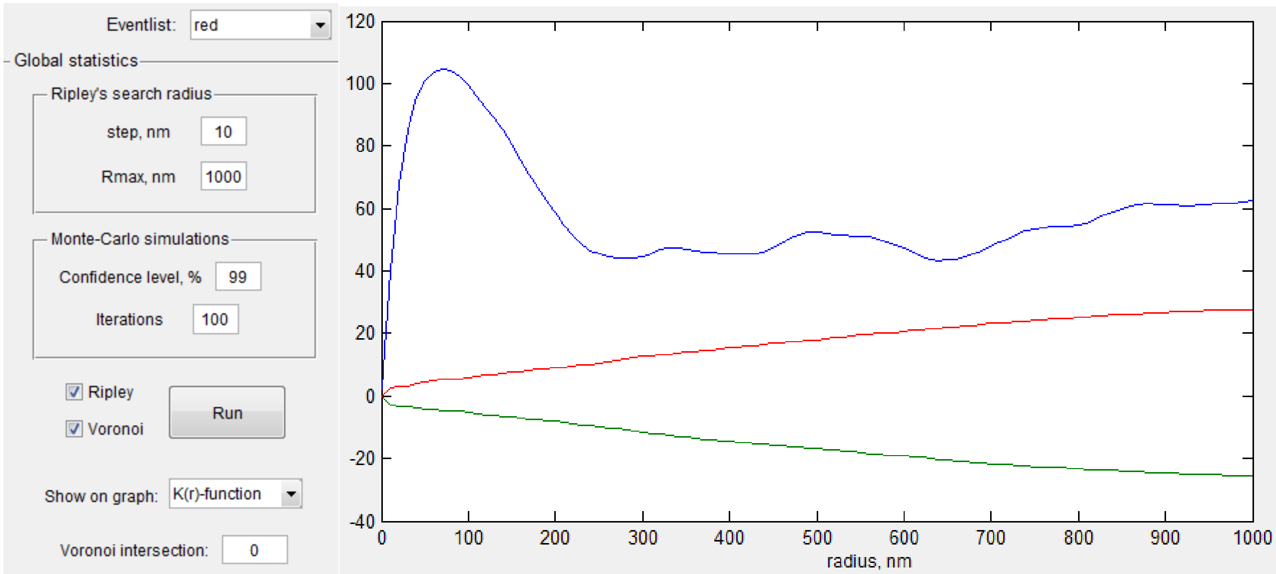
You can change pixel size (default 20 nm) and brightness (multiplier, default 3). Choose desired method for creation of ROI. The following methods available: freehand (default, the most flexible), polygon, rectangle, ellipse. Press "Create mask": you will be able to draw the outline of you ROI directly in the preview window. After finishing drawing, click twice inside the region, the preview will update showing only the data in the selected ROI. If not satisfied, you can press Reset and select a ROI again.

After successful creation of ROI, mask area and number of events will be shown.



Calculation of Ripley functions and Voronoi polygons statistics

Go to panel “Global statistics”. Enter desired step and maximum search radius for Ripley’s functions. Enter desired confidence level and number of iterations for Monte-Carlo simulations. If you do not wish to make Monte-Carlo simulations, put 0 as number of iterations. Check with statistics you would like to calculate (Ripley and/or Voronoi). Press “Run”. After end of calculations, you can select different graph to display (Ripley’s K, L, L-r, g or areas of Voronoi cells). In “Voronoi intersection” will be shown the area of the polygon corresponding to the intersection between the experimental graph and the graph of the average values obtained from random data with the Monte-Carlo simulation (this intersection value can be used for thresholding later).



Cluster density maps

Go to panel “Cluster map”. Choose method for reconstruction of the map. Methods available:

L(R) – the density at each localization point will be calculated as Ripley’s L-function at radius R, with subsequent interpolation on pixels of chosen size;

g(R) – the density at each localization point will be calculated as pair correlation function at radius R with dr equals the step for Ripley’s analysis from the previous section, with subsequent interpolation on pixels of chosen size;

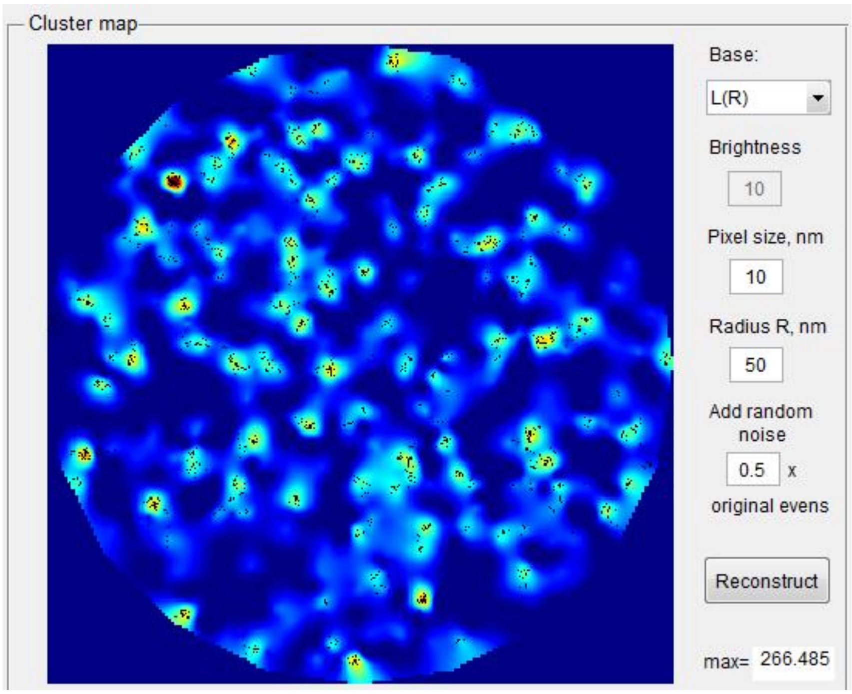
Gaussian – the map will be built as a histogram image of chosen pixel size, filtered by a Gaussian filter with σ= R;

Voronoi diagram – the diagram will be shown, using localizations as seeds;

Voronoi density map – the density at each localization point will be calculated as inverse value of the corresponding Voronoi polygon area, with subsequent interpolation on pixels of chosen size, the obtained values are then normalized and multiplied by “brightness” value to make the dynamics of the picture better fit that of display.

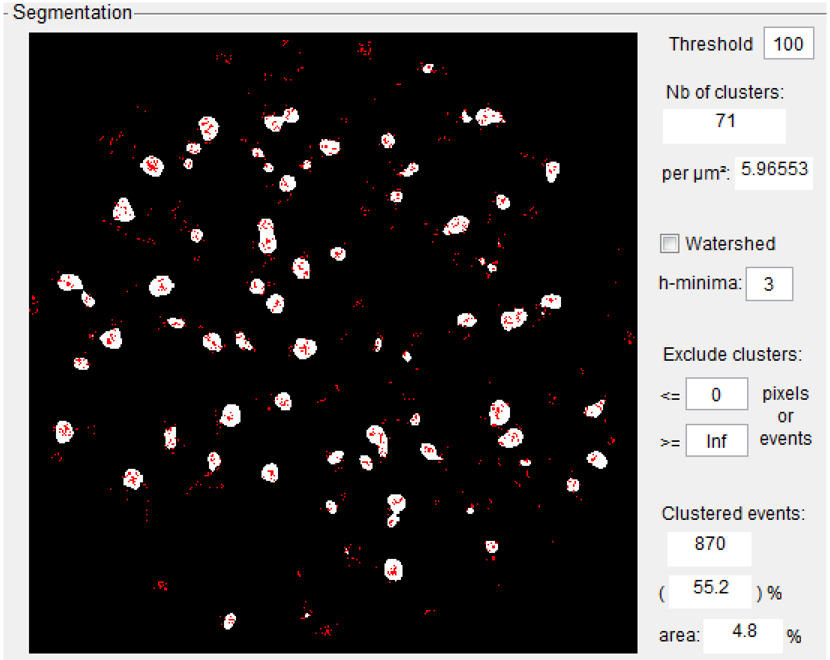
You can add uniformly distributed localizations to you data by putting “Add random noise” (available for L(R) and g(R)). While not affecting much the result, it helps with interpolation of local densities to the grid of pixels. The number of random points should be chosen as small as possible (0.5 or 1 times the number of the original localizations can be OK).

After setting up the parameters, press “Reconstruct”. After end of calculation, the cluster map and the maximum obtained value (may help to choose a threshold later) will be shown. The original data points are shown as black pixels.



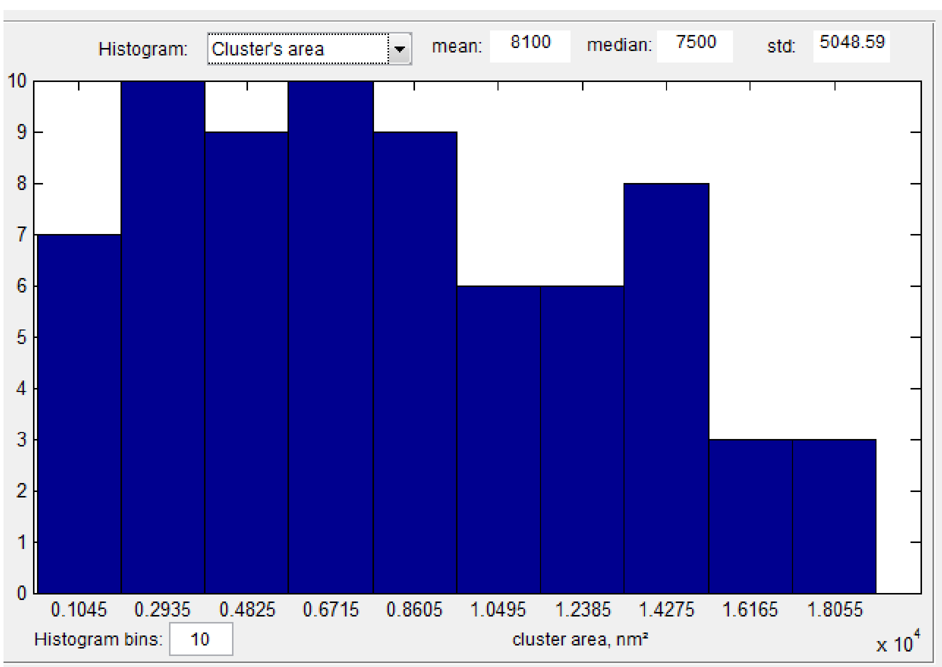
Segmentation

You will be able to segment the reconstructed density map. Proceed to panel “Segmentation”. You should choose a threshold for binarization. In case of segmentation by Voronoi diagram, it will be set automatically to the intersection (see above “Calculation of Ripley functions and Voronoi polygons statistics”). Regions with density higher than the threshold (clusters) will adopt values of one, the rest (background) will adopt values of zero. The data in this panel update automatically after you change any parameter. You see different statistics, as number of detected clusters, their density, number of clustered events, percentage of area occupied by clusters. Additionally, you can make “watershed” transform with parameter “h-minima”; it will help to separate clusters, sticking together (not available for Voronoi diagram mode). You can also exclude too small or too big clusters by imposing the limits on size in pixels or on number of events in cluster (for Voronoi diagram mode).



Cluster properties

After segmentation, you can have statistics on different cluster properties (area, equivalent diameter, eccentricity, number of localizations in cluster, density of localization in cluster). You can see histograms of the corresponding values in the lower right corner of the window. In the upper part of this panel you see mean, median and standard deviation of current variable. In the lower part you can change number of histogram bins.



Save data

You can save all the data automatically from menu “File – save everything in a folder”. Choose the folder in the newly opened window. The data will be saved in the following filenames:

preview image: ‘prev_channel_pixsize_brightness.tif’;

K(r), L(r), L(r)-r, g(r) graphs: ‘type_channel_step_Rmax_confidence_iterations.png’;

Voronoi cell sizes: ‘Vor_channel_confidence_iterations_intersection.png’;

segmented images: ‘segm_type_channel_brightness_pixsize_R_noise_threshold.tif’;

Voronoi diagram: ‘VorDiagram_channel.png’;

density maps: ‘type_channel_brightness_pixsize_R_noise_maxValue.tif’;

histograms of cluster statistics: ‘type_channel_mean_median_std.png’;

table of cluster properties: ‘clusterStat_channel.ascii’.

The latest table contains one localization in each row, with its values of area (nm^2), equivalent diameter (nm), eccentricity, number of events, density of events ($1/(1000*nm^2)$), in each column, correspondingly. The variables will be replaced by corresponding values.