## Installation

Download and run "ClusterViSu3D_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.

Running ClusterViSu3D

Run "ClusterViSu3D.exe" (default path: "%ProgramFiles%\ClusterViSu3D\application\", could have been changed during installation). The main window of the program will appear. The window was optimized for the screen resolution 1920x1080 pixels; some components can get hidden if viewed on a smaller screen.
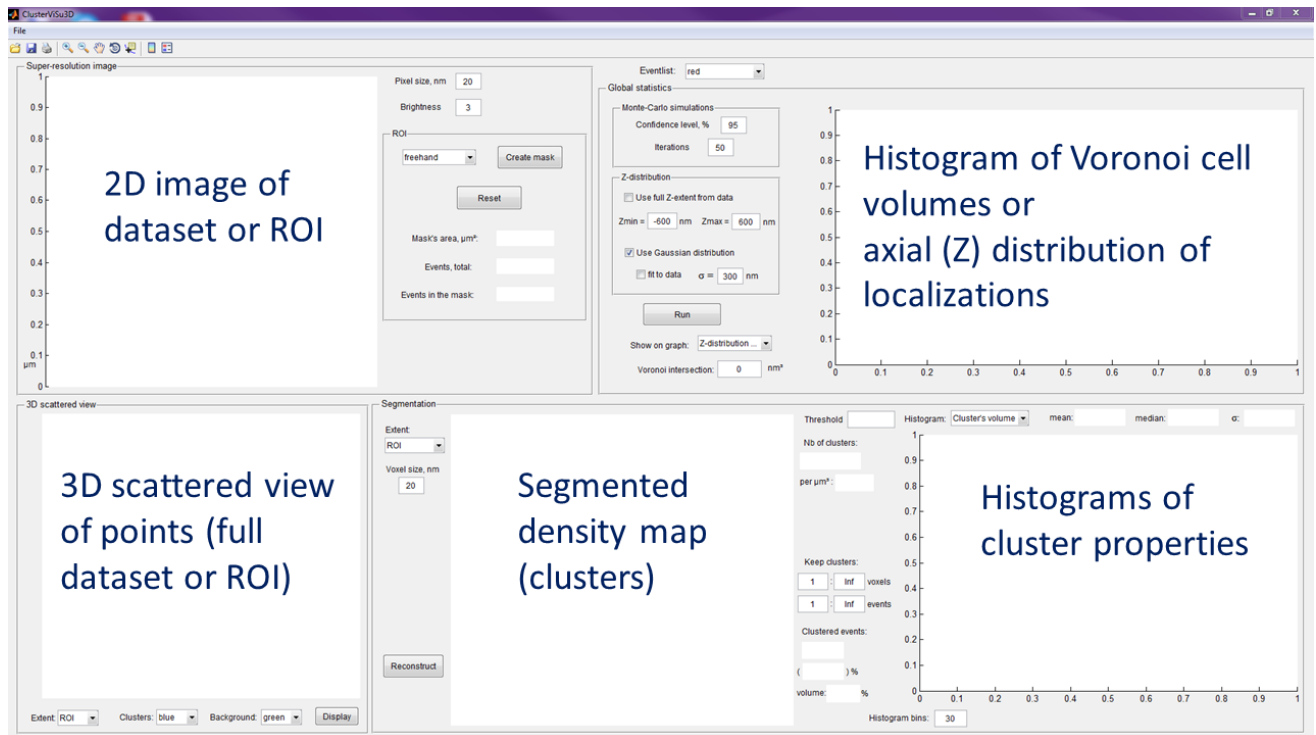
Loading a localization table

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

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

Press "Browse…" and choose a localization table file.

For the test data 'H2Btruncated.ascii', choose the 'SharpViSu' format.

Selection of a region of interest (ROI)

Here you can create a ROI for the X-Y coordinates, the Z-extent can be set in the next step. Your data will be shown as a projection on the X-Y plane. You can change the pixel size (default 20 nm) and brightness (default 3). Choose a desired method for creation of ROI. The following methods are available: freehand (default, 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, the mask's area and the number of events will be shown.

For first tests, choose a small region in order to improve speed of further calculations.
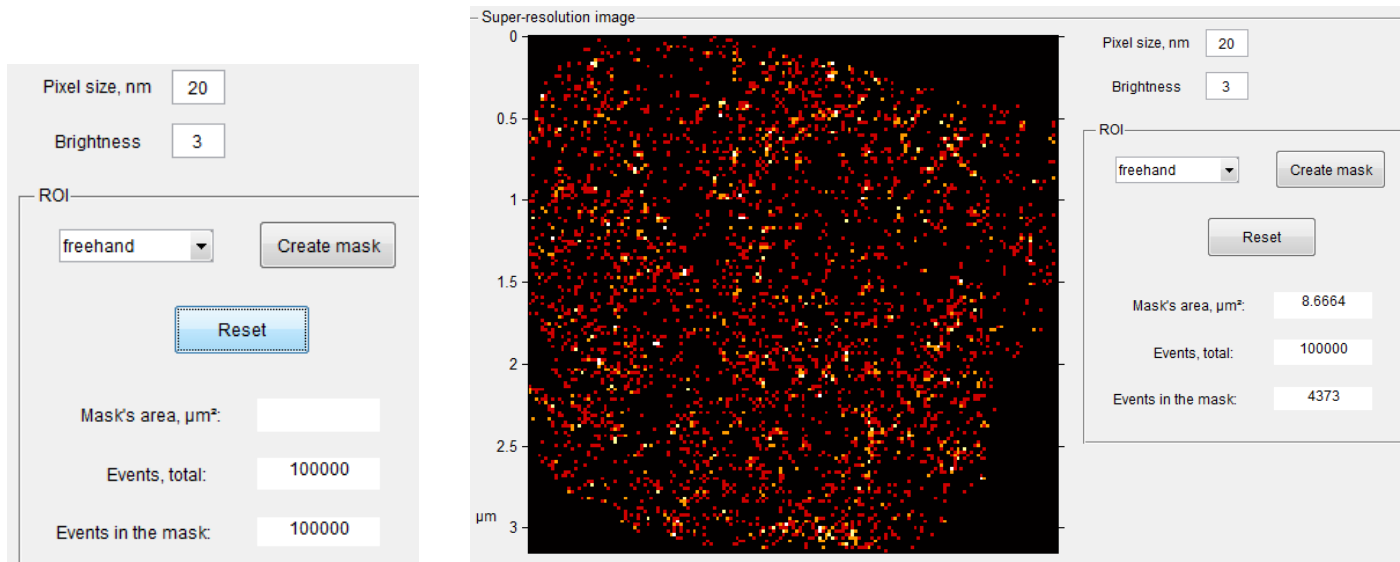
Calculation of Voronoi polygon statistics

Go to the panel "Global statistics". First, determine the Z-range of your data (the actual Z-profile of the dataset can be shown with the "Show on graph" menu). Any data points outside the [Zmin, Zmax] range will be removed and the randomized distributions for Monte-Carlo simulations will have the axial extent from Zmin to Zmax. If you choose "Use full Z-extent from data" Zmin and Zmax will be determined as the minimal and the maximal Z-values of the data points.

If your data has stronger localization density in the middle of the Z-range (typical situation for 3D experiments with astigmatism) it is convenient to approximate this density with a normal distribution. To do so, check "Use Gaussian distribution". You can determine the s.d. of the normal distribution directly from the dataset (use "fit to data") or you can put an *a priori* known value under "σ =" (it can be e.g. the Z-width of the point spread function). For the test data, you can keep all the parameters by default.
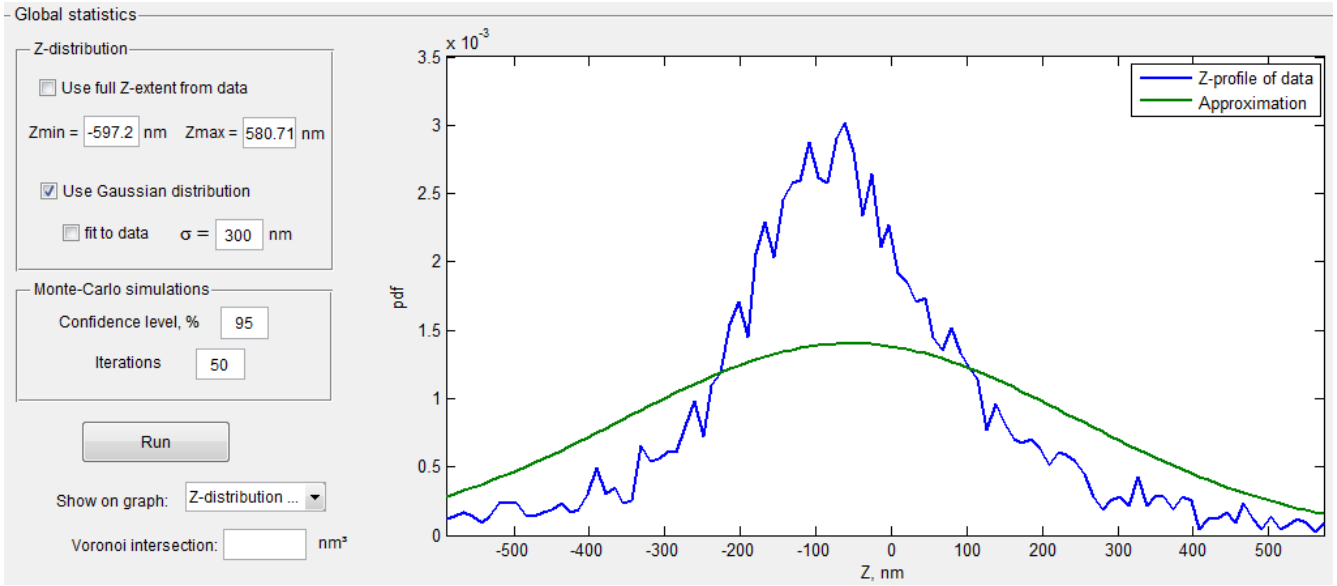
Enter a desired confidence level and a number of iterations for Monte-Carlo simulations. If you do not wish to run Monte-Carlo simulations, put 0 as number of iterations. For first tests you can use a lower number of iterations (5-10) for speed. Press "Run". After the end of the calculations, you can select the "Voronoi cell volumes" graph to display. The volume of the polygon corresponding to the intersection between the experimental graph and the graph of the average values obtained with the Monte-Carlo randomizations will be shown in "Voronoi intersection" (this intersection value will be used for a thresholding later).
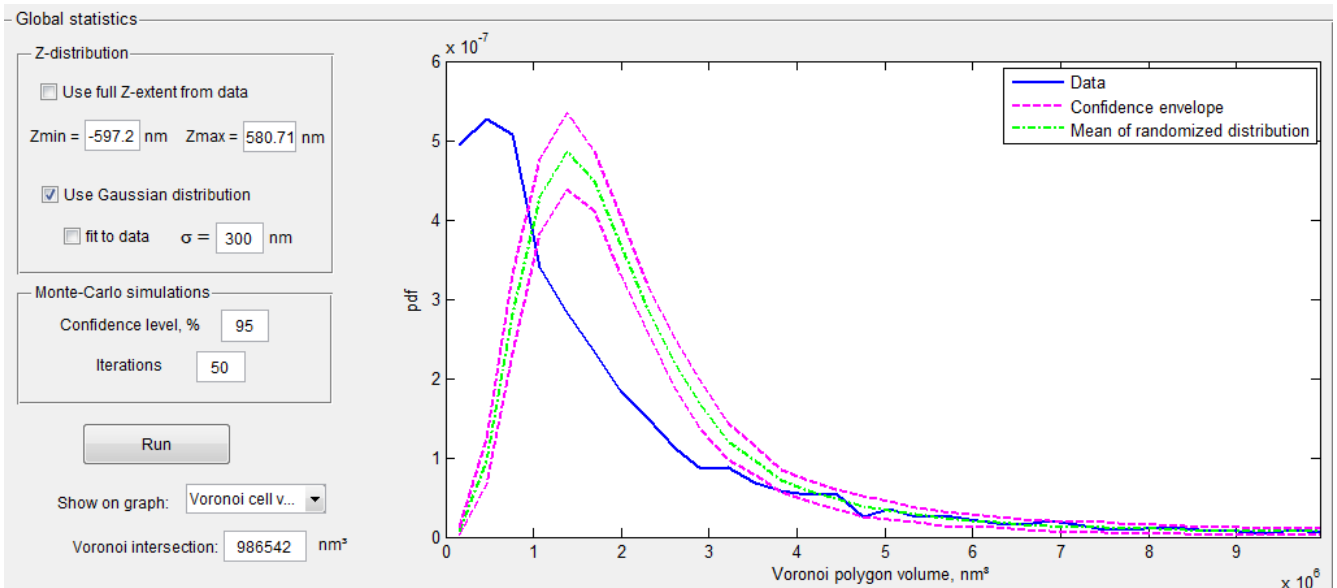
3D scattered view

Go to the panel "3D scattered view". You can choose the region to show (the ROI or the full dataset) and the color for the clustered and the background points. The points will be assigned to clusters or to the background based on the threshold determined at the previous step.
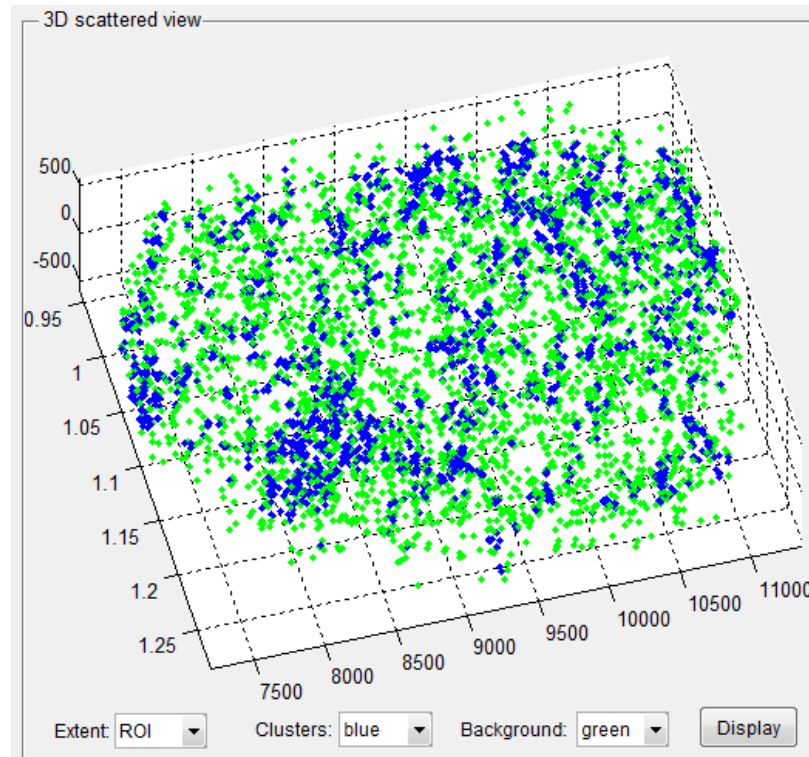

3D density map and segmentation

Go to the panel "Segmentation". Choose the region and the voxel size for the density map calculation and segmentation. Attention, processing of volumes with high number of voxels can be very slow (e.g. a few hours for $5 \cdot 10^7$ voxels), so for first tests use a smaller ROI or a bigger voxel size. Press "Reconstruct". There is no progress bar for this function, please be patient. After the end of the calculations, the segmented volume will appear automatically. You can rotate the 3D view using the "Rotate 3D" tool in the top toolbar.

Here you can also save the density map in the MRC format (a common format for cryo-electron microscopy) and the localization tables in a text format with the x,y,z coordinates in the 4,5,6[th] columns and the local densities in the 10th column. The threshold value will be added to the file names.
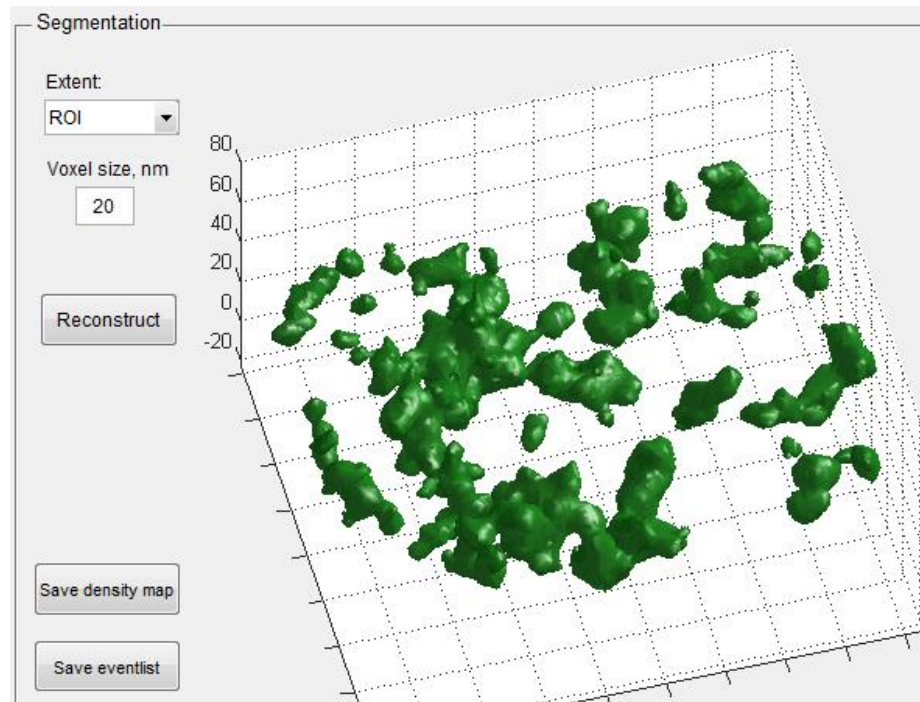
In the middle of the panel there are controls for segmentation. The threshold for segmentation was determined automatically from the Monte-Carlo simulations, but you can also use another value. You can also set limits on the volume of clusters (number of voxels) and on the number of events in clusters. Some general information about the clusters, such as their number, density, number of localizations is displayed.
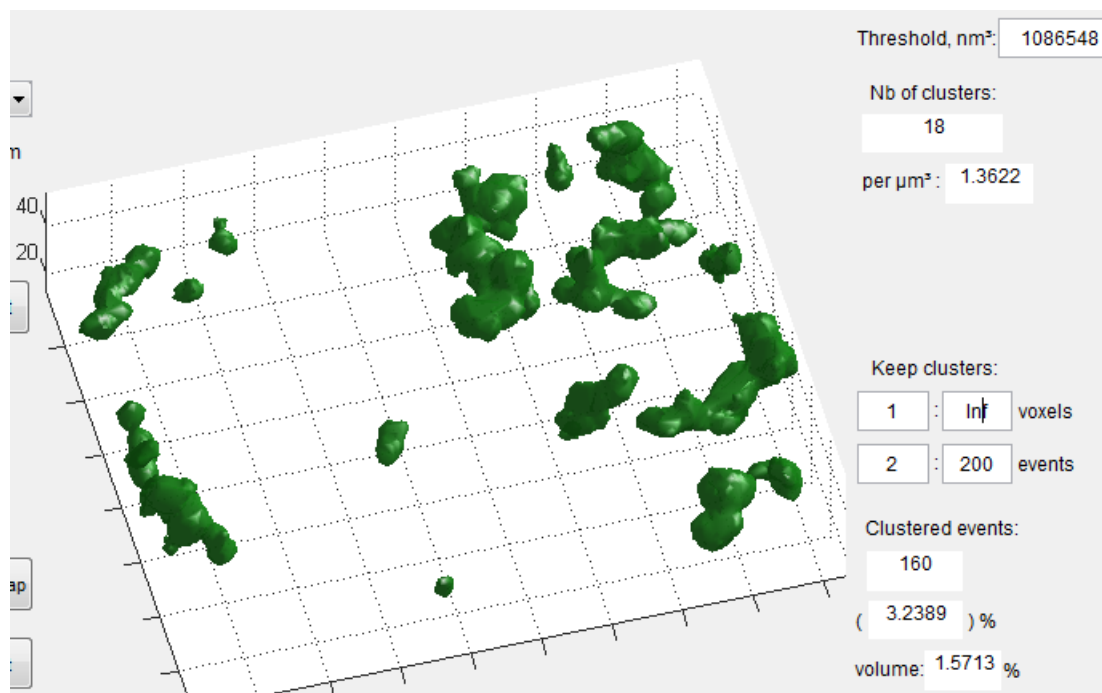
In the right part of the panel you can see statistics on detailed properties of the determined clusters, such as the volume, the diameter, the number of localizations in a cluster and the density of localizations in a cluster. You can change the number of the histogram bin to improve the representation.
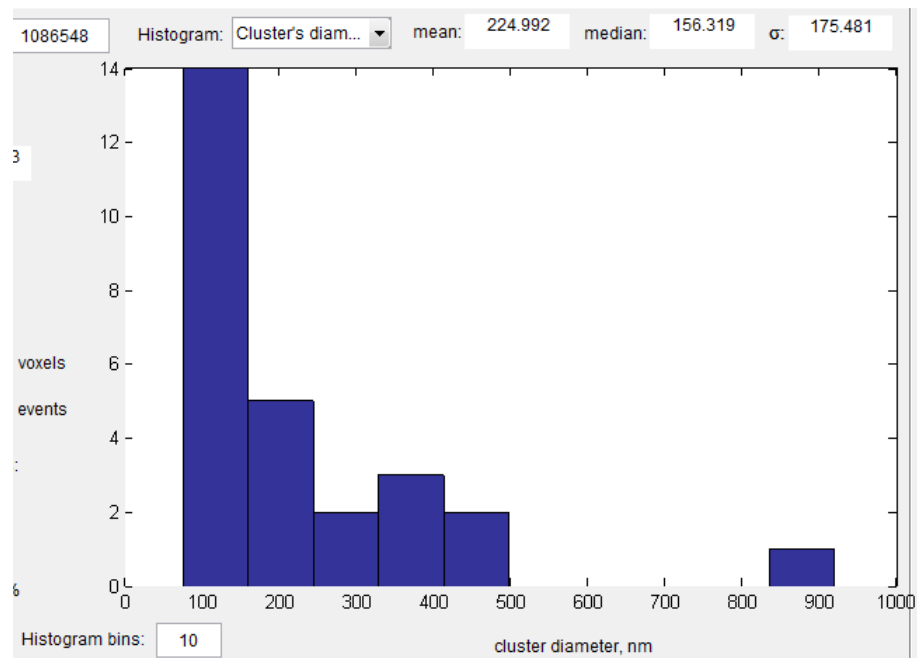
Saving the data

This feature is under development. You can use menu "File - save everything in a folder". Choose the folder in the newly opened window. The 2D preview and the screenshot of the whole window will be saved in the folder.
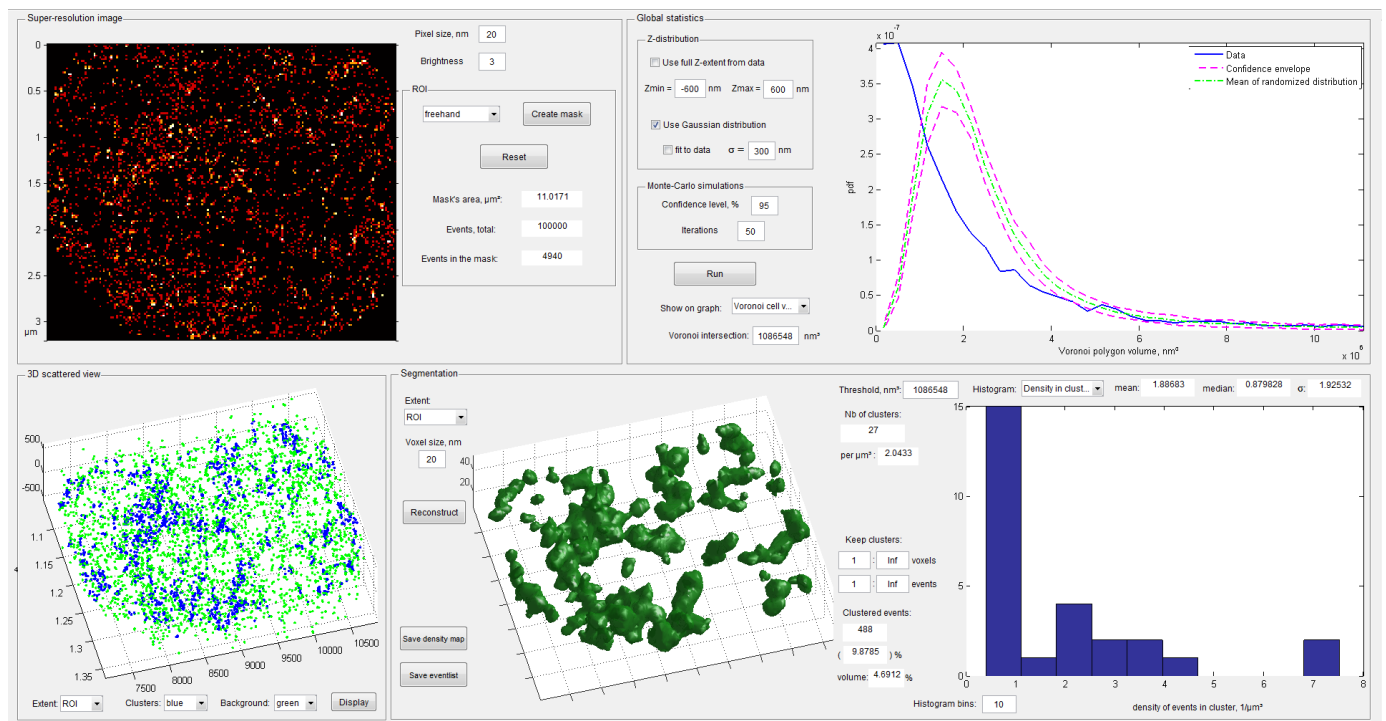
The test data "H2Btruncated.ascii" is the histone H2B dataset used in the manuscript, truncated to have only 10^5 points to improve the speed of calculations.