User's guide of DecodeSTORM

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1. Requirements

Operation system: DecodeSTORM has been tested on Windows 7 (64-bit), Windows 10 (64-bit).

Software: ImageJ or FIJI.

Note: please set at least 4 GB memory buffer for ImageJ.

2. How to install

2.1 How to configure Java in Windows

If the Java environment variables are already configured, skip this step.

Java download address: https://www.oracle.com/technetwork/java/javase/downloads/index-jsp-138363.html, Select 64bit to Download.

Installation: We installed java 1.8 in Windows, of course, you can install other versions, but make sure ImageJ works. Double-click the Java 1.8 installation package → Install the JDK: Next → Select the installation location and remember, Next → Install the JRE: Select the installation location and remember(Installed in the same folder as the JDK), Next → Close.

Set environment variables:

1) This PC → Properties → Advanced system settings → Environment Variables.

2) Environment Variables → System variables → New → Variable Name: JAVA_HOME. Variable value: JDK Installation Path (for example, D:\Program Files\Java\jdk1.8.0_271) → OK.

3) System variables → CLASSPATH (If no, new one) → Variable value: .;%JAVA_HOME%\lib\dt.jar;%JAVA_HOME%\lib\tools.jar; → OK.

4) System variables → PATH → Open → New → Input: %JAVA_HOME%\bin → New → Input: %JAVA_HOME%\jre\bin → OK.

5) Open the command prompt, input Java -version, if the version information is displayed, the configuration succeeds.

2.2 How to Install ImageJ or FIJI

ImageJ download address: https://imagej.en.softonic.com/

FIJI download address: https://fiji.sc/, Select 64bit to Download.

Installation: After downloading, unpack it and use it (double-click ImageJ-win64.exe).

2.3 How to install DecodeSTORM

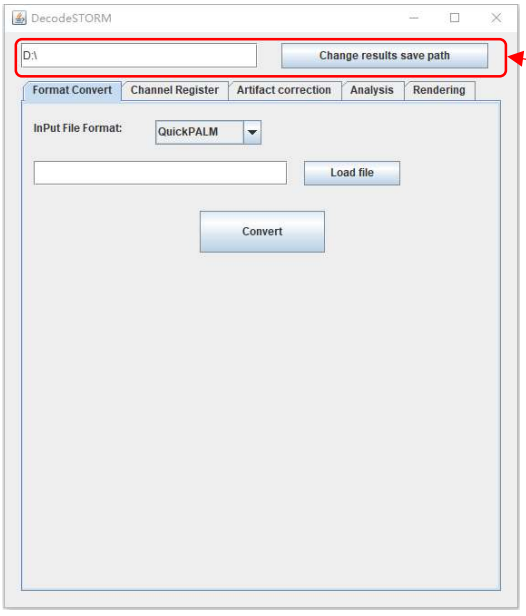
DecodeSTORM is built for ImageJ independently. To install, simply copy three dynamic link library (libopenblas.dll, opencv_world450.dll, DecodeSTORM_CPPDL-L.dll) files into the ImageJ installation folder. And then copy the corresponding .jar plug-in file to the plugins folder of ImageJ.

3. How to use

Open the DecodeSTROM: ImageJ → Plugins → DecodeSTORM

3.1 Change results save path

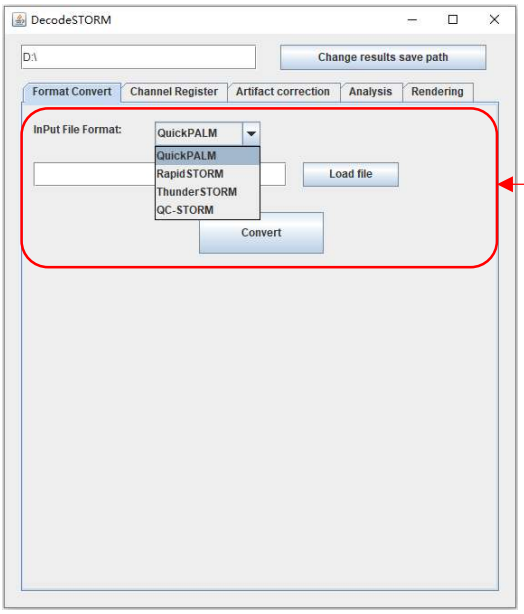
Click the "Change results save path" button to change the save path of format convert, Artifact correction, and quantitative analysis results. The default save path is D:\.



Change the save path of format convert, artifact correction and quantitative analysis results

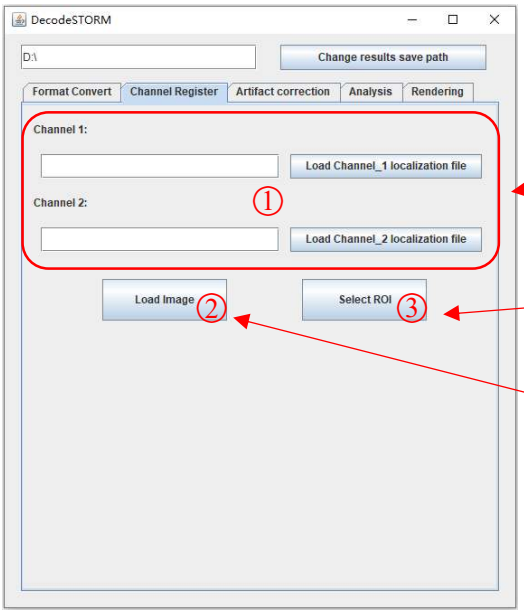
3.2 Format Convert

Format convert module is used to converts the localization table generated by QuickPALM, RapidSTORM, ThunderSTORM, or QC-STORM into the format suitable for DecodeSTORM. Results are saved at the selected path.



Format convert to get the localization table suitable for DecodeSTORM.

3.3 Channel Register



Load the localization tables.

Select ROI to artifact correction and quantitative analysis.

Load data scatter image.

Load localization file: DecodeSTORM has double color channels: channel 1 (green) and channel 2 (red). A single localization table can be load either channel. Double localization table (double color data) should be load double channels.

Load image: Clicking the "Load Image" button will generate a scatter image of the data when the localization table is in the channel.

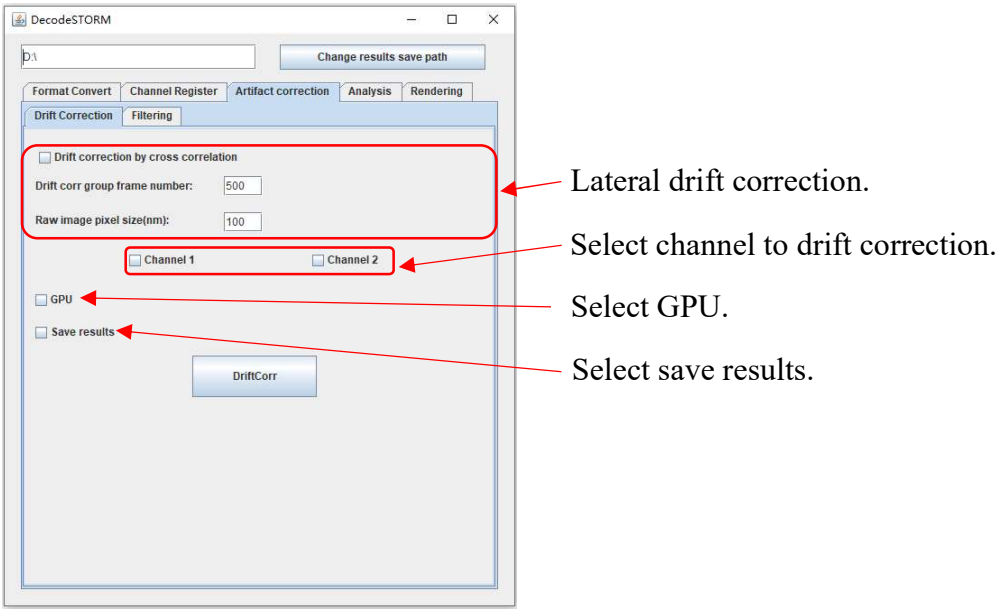
Select ROI: Select ROI for artifact correction and quantitative analysis. First, Click the ImageJ “Rectangle” button to select the ROI area. Then, to use the keyboard shortcut Ctrl+T to load the ROI into the ROI manager and select. Lastly, click the “Select ROI”

button of DecodeSTORM. DecodeSTORM allows the user to select multiple ROI areas to load into the ROI manager, and the user can select one of them for ROI artifact correction and quantitative analysis.

3.4 artifact correction

3.4.1 Drift correction

Drift correction: DecodeSTORM provides redundant cross-correlation drift correction to correct artifacts caused by sample drift. Users need to set the number of drift correction group frames (default: 500) and the original image pixel size (default: 100), then select one or all channels for drift correction and save the results. Note that subsequent modules will continue to provide options for channel selection and whether to save the results. We also offer a GPU option for accelerated drift correction (the computer must have a Cuda-enabled GPU).



3.4.2 Filtering

Imprecise localizations, isolated localizations, duplicated localizations in the same frame, molecules reappearing in subsequent frames result in imaging artifacts, which might impact quantitative analysis results, so the localizations need to be removed or merged.

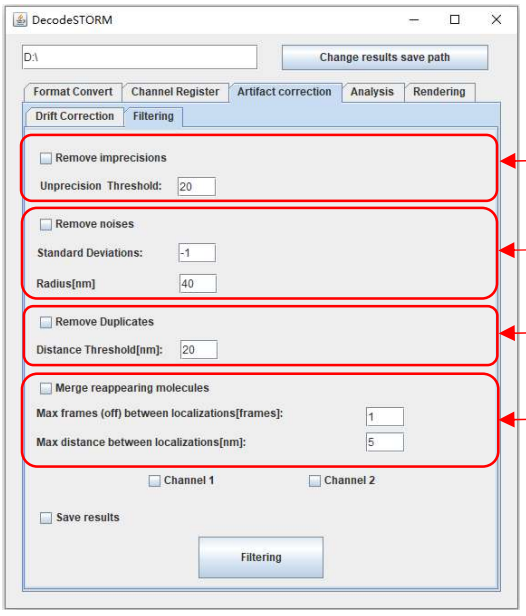
Remove imprecise localizations: This function requires localization tables to have localization precision or to be calculated using other information. Users need to set an

imprecise localization threshold (typically 20) to remove imprecise localizations.

Remove isolated localizations: Remove isolated localization based on local density. The parameters set by users are the radius to determine the size of the area to search for neighbor localization events and the standard deviation to determine the minimum number of localization events within the radius (default radius 40, stdev -1).

Remove duplicated localizations in the same frame: Duplicated localizations are determined according to the euclidean distance between localizations in the same frame. DecodeSTORM offers a distance threshold (typically 20nm) to remove duplicated localizations.

Merge molecules reappearing in subsequent frames: Reappear molecules are determined according to the euclidean distance between localizations in the subsequent frame. In DecodeSTORM, users need to set 2 parameters to merge molecules reappearing in subsequent frames: max frames of molecules reappearing (typically 1) and max distance between localizations (default 5nm).



Remove imunprecise localizations.

Remove isolated localizations.

Remove duplicated localizations in the same frame.

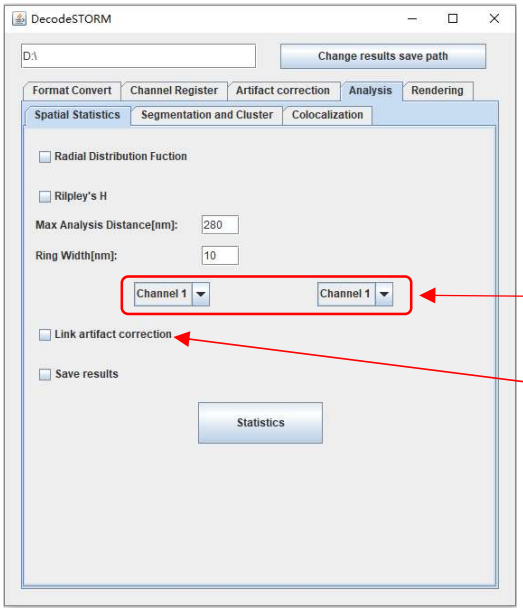
Merge molecules reappearing in subsequent frames.

3.5 Quantitative analysis

3.5.1 Spatial statistics

In the spatial statistics module, the max analysis distance (default 280nm) and ring width (default 10nm) need to be set. Selecting the same channel means calculating the self-clustering of the channel data, and selecting different channels means the co-

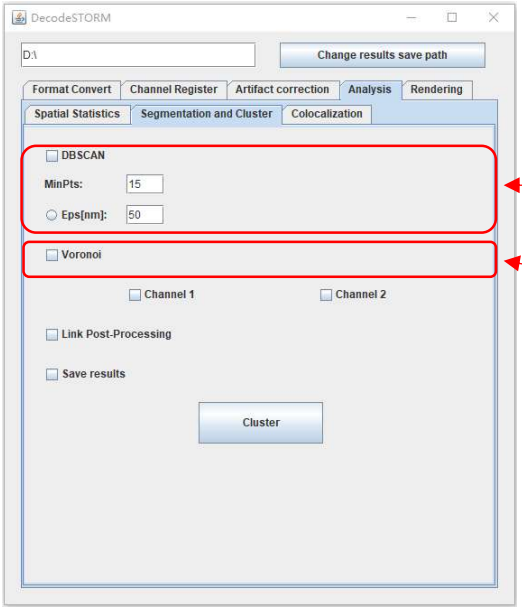
clustering of the two channels. Radial Distribution Function (RDF) and Ripley's H Function generate their function graphs when they are used. At the same time, the user can choose to link to the artifact correction module to use the corrected data or to use the raw data. Note, the subsequent modules in the "Analysis" also provide "Link artifact correction".



Select channel to calculate self-clustering or co-clustering.

Select link artifact correction.

3.5.2 Segmentation and Cluster



Density-based spatial clustering of applications with noise.

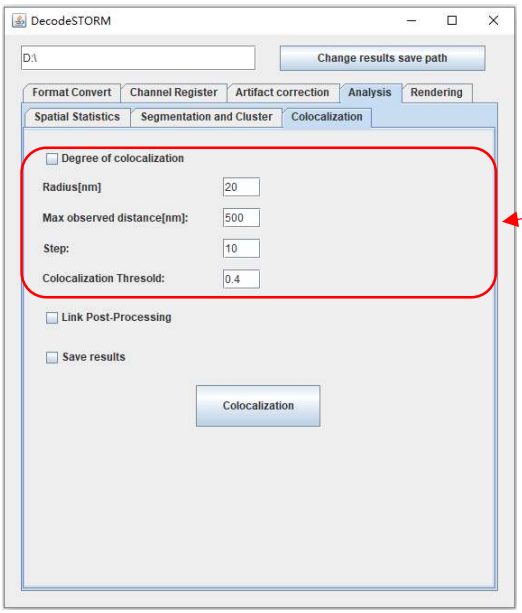
Voronoi Tessellation

DBSCAN has two parameters: the radius *eps* (default 50nm) that determines the size of the search nearest neighbor localization event region and the minimum number of localization events *MinPts* (default 15) in the region, which generates the molecular

cluster and segmentation maps and gets the number of clusters. In DecodeSTORM, *eps* can also be calculated automatically. Voronoi can cluster molecules to generate Voronoi Diagram without setting any parameters.

3.5.3 Colocalization

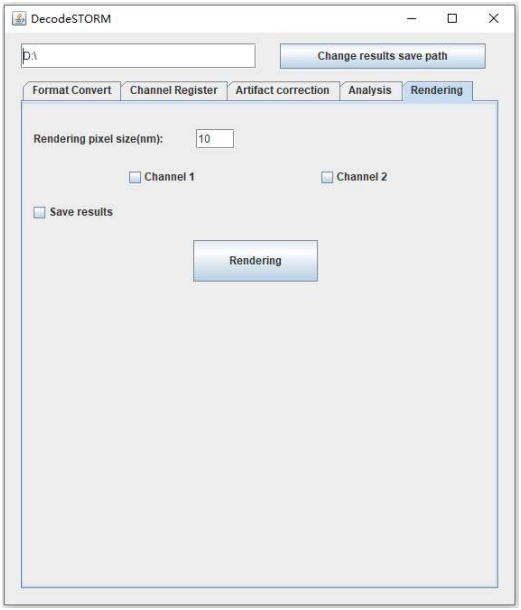
In DecodeSTORM, colocalization analysis is performed using Clus-Doc with four parameters to be set: search radius r, maximum observation distance R_{max}, search step *Step* and CBC threshold. The CBC threshold allows the user to autonomously segment colocalization and uncolocalization localizations. Colocalization analysis using this method produces histograms of CH1 → CH2 and CH2 → CH1 degree of colocalization score.



Degree of colocalization from Clus-Doc

3.6 Visualization

In addition to the generation of the scattered map, DecodeSTORM provides Gaussian rendering for SMLM data visualization, and the user needs to set the rendering pixel sizes, and raw image pixel size (in Drift Correction module of Artifact correction) to generate a suitable rendering image.



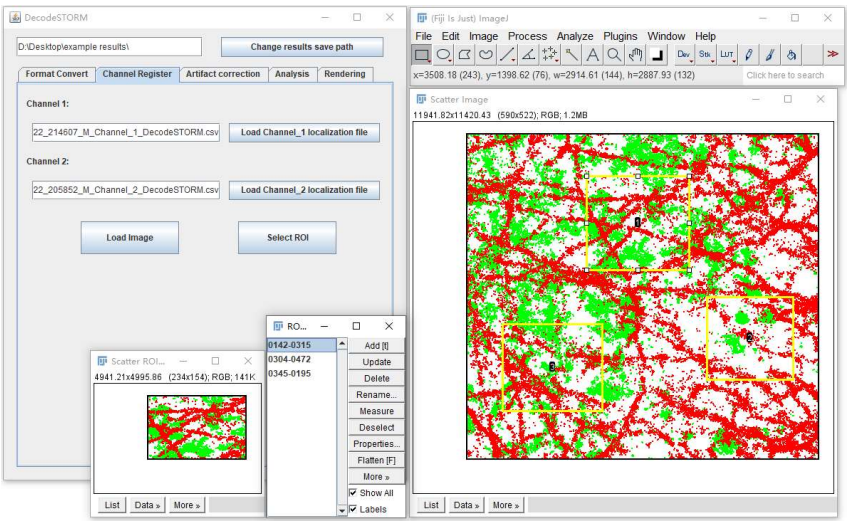
4. Note

The default parameters in DecodeStorm are set based on our test data. "Save results" is selected when using the “Filtering”, “SpatialStatistics”, “Segmentation and Cluster”, “Colocalization” and “Rendering” module, the corresponding folders (Name: “FilteringResults”, “SpatialStatisticsResults”, “SegmentationAndClusterResults”, “RenderingResults”, “ColocalizationResults”) will be generated for saving these results. These folders are located at the selected save path, drift correction and format convert results are located at the same path. In addition, the numbers in the CSV file generated by DecodeSTORM are presented in scientific notation.

5. Example

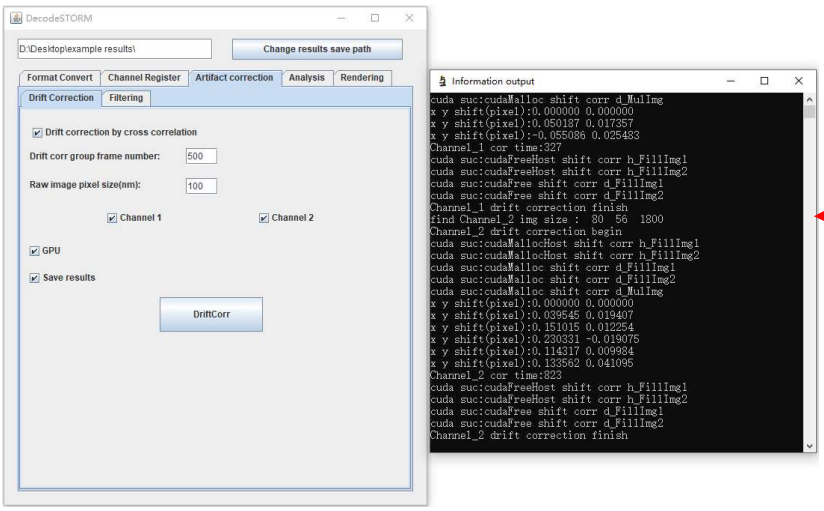
We provide double-color data for testing. The data were labeled microtubules and mitochondria in U2OS cells with AF647 and CF568 respectively, and diffraction-restricted image sequences were obtained by SMLM, then obtained by QC-STORM localization. The ROI we selected was also saved, and after importing the image, the saved ROI is loaded for testing.

Load localization file → Load Image → ”Ctrl + T” → ROI Manager → More → Open → Open 0142-0315.roi → Click on the selected → Select ROI. specific operation results are shown in the following figures.



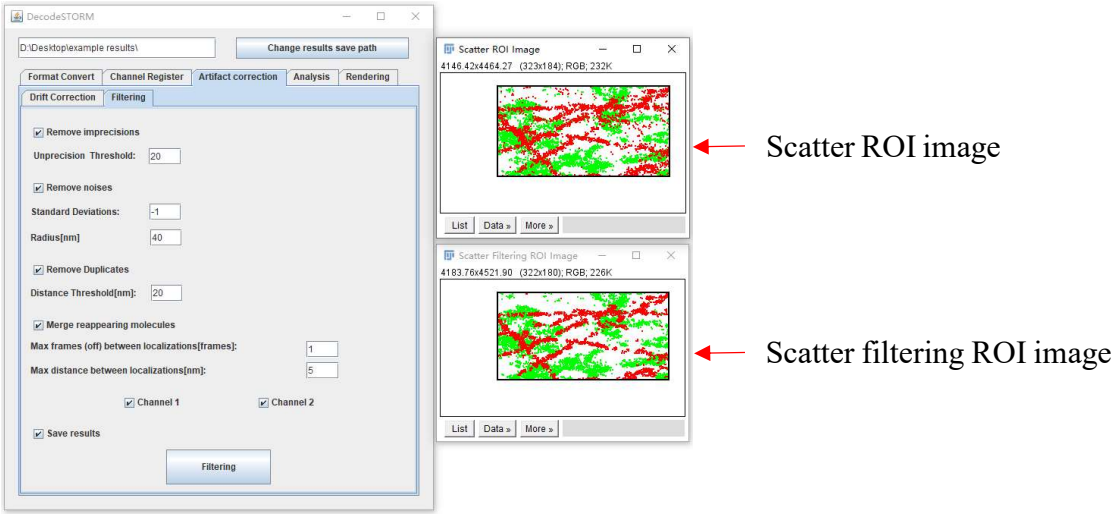
5.1 Artifact correction

5.1.1 Drift correction



Drift correction information

5.1.2 Filtering

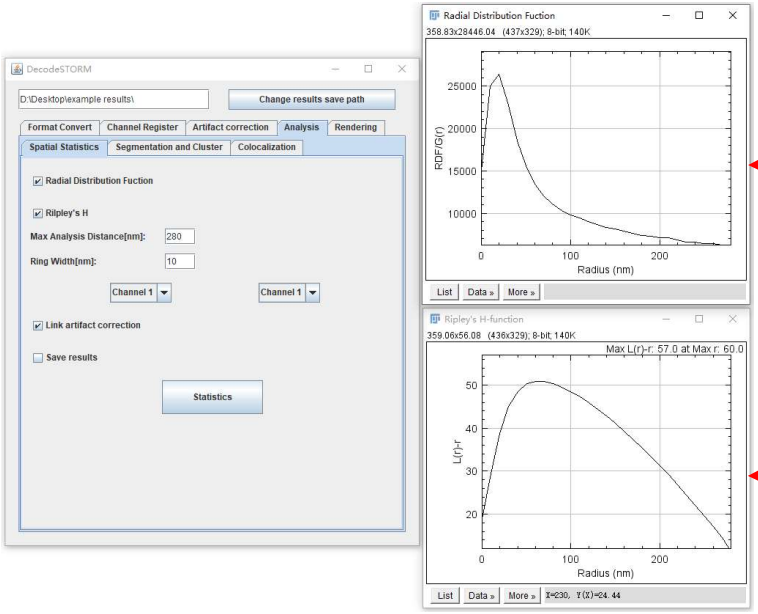


Scatter ROI image

Scatter filtering ROI image

5.2 Quantitative analysis

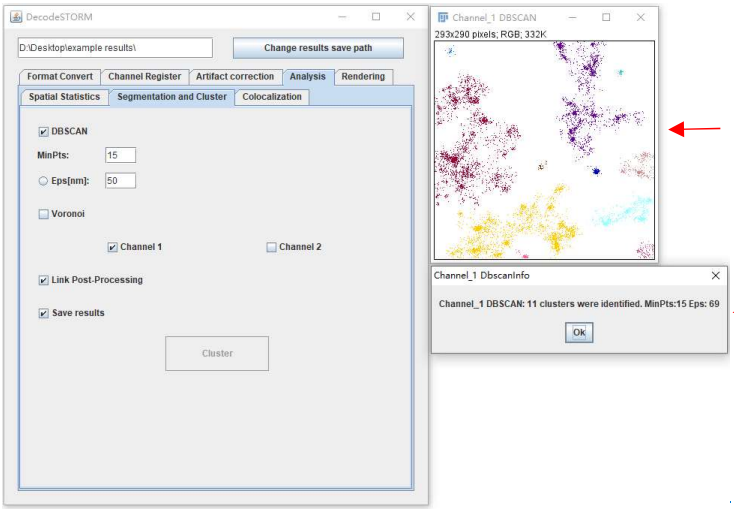
5.2.1 Spatial Statistics: RDF and Ripley's H Function



Channel 1 Radial distribution function

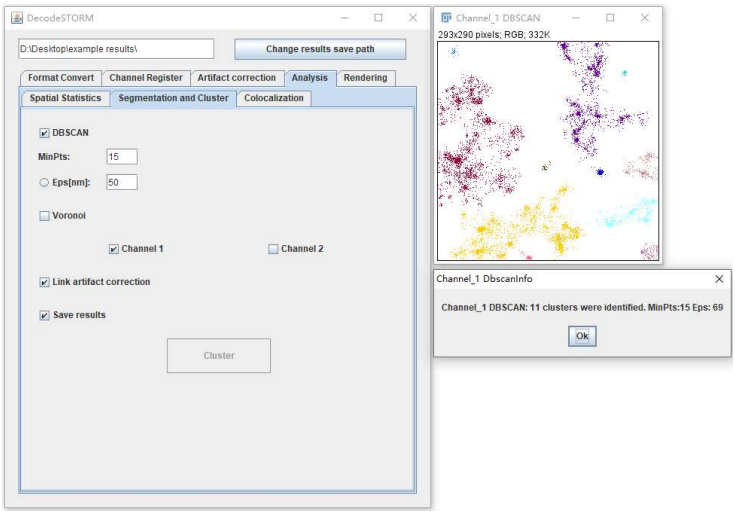
Channel 1 Ripley's H function

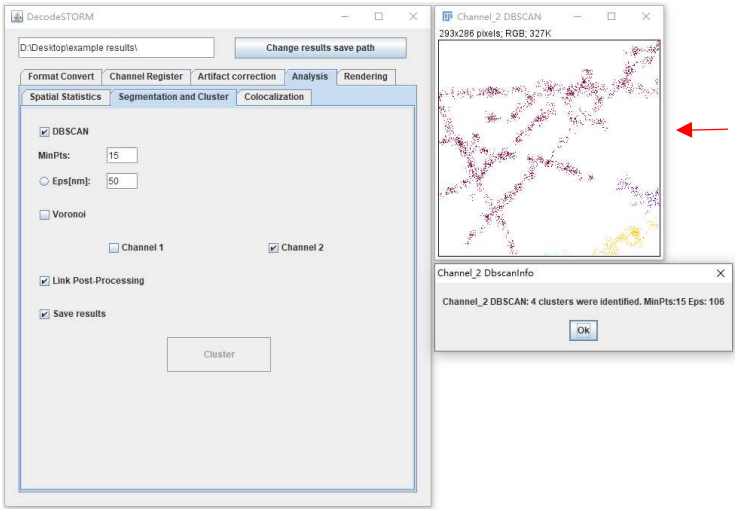
5.2.2 Segmentation and cluster: DBSCAN and Voronoi



Channel 1 DBSCAN image

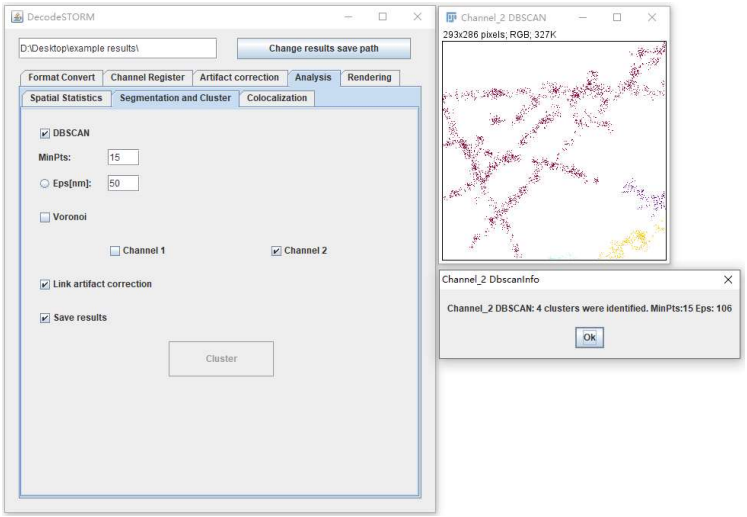
Channel 1 DBSCAN information

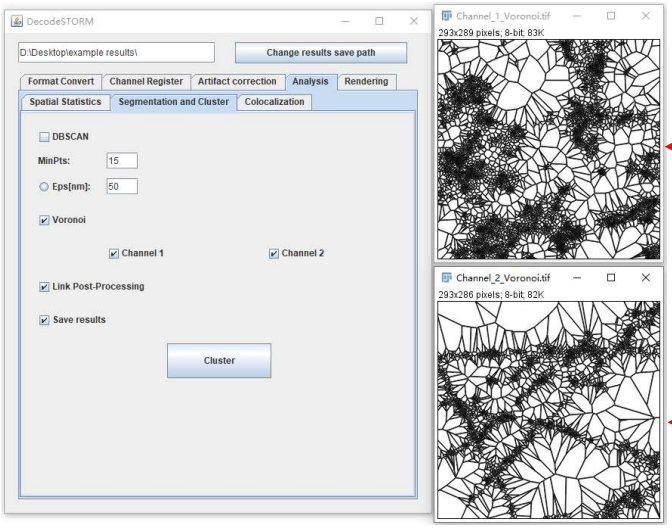




Channel 2 DBSCAN image

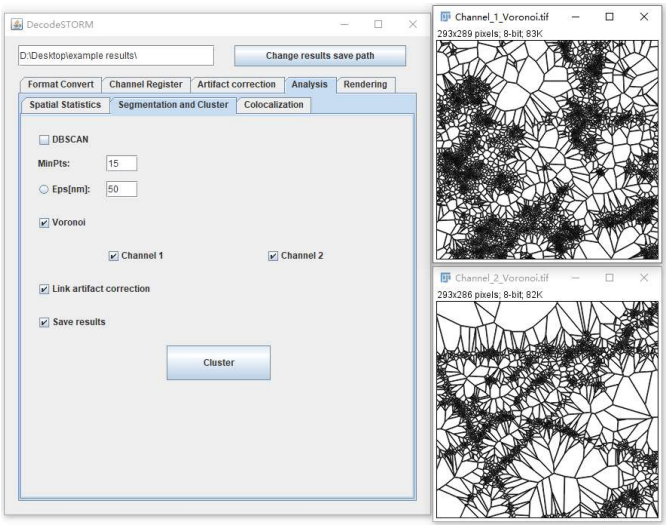
Channel 2 DBSCAN information



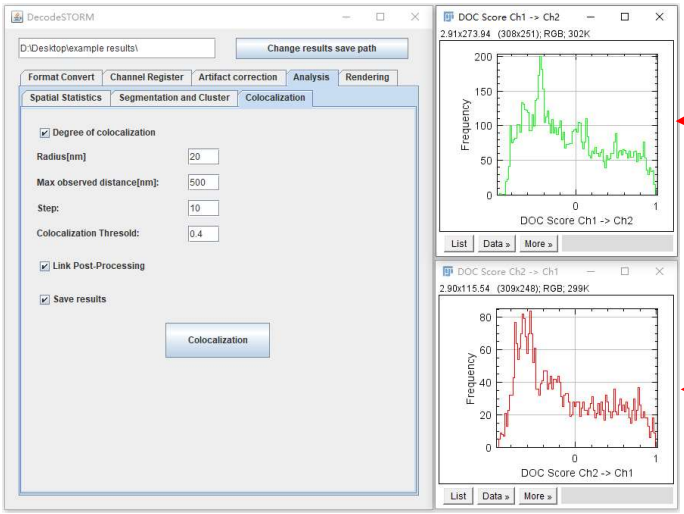


Channel 1 Voronoi diagram

Channel 2 Voronoi diagram

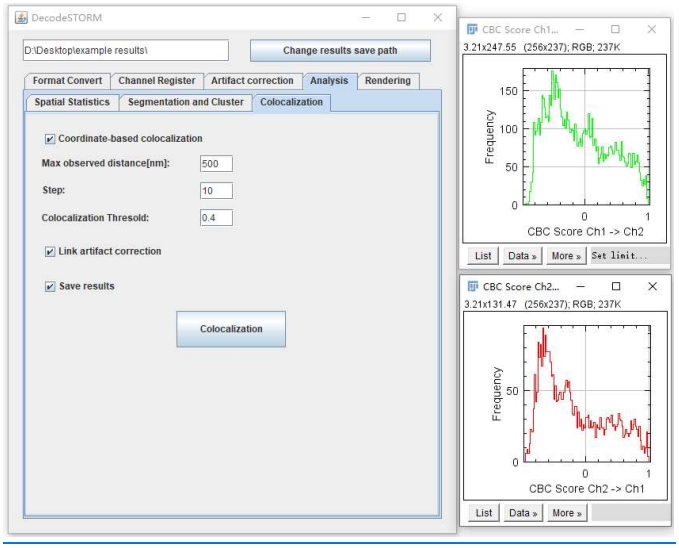


5.2.3 Colocalization: DOC

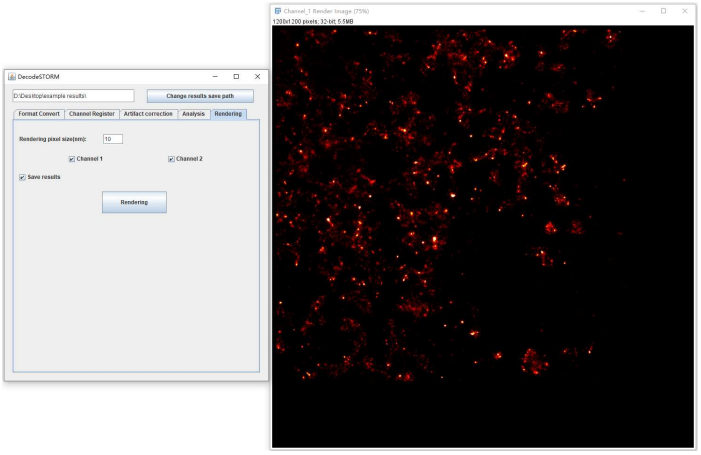


Histograms of CH1 → CH2 degree of colocalization score

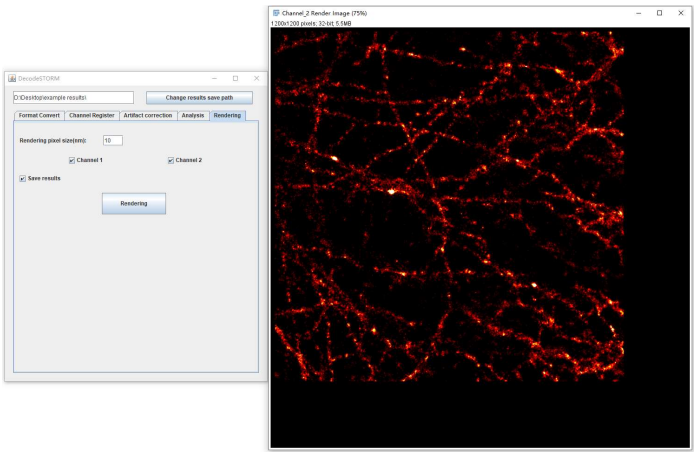
Histograms of CH2 → CH1 degree of colocalization score



5.3 Visualization: Gaussian render



Channel 1 render image



Channel 2 render image