User’s guide of DecodeSTORM

June 8, 2022

Contents

1. Requirements

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

Software: ImageJ.

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

2. How to install

2.1 How to install ImageJ

Users can download ImageJ at https://imagej.en.softonic.com/. After downloading, double-click "ImageJ-win64.exe" to install.

Of course, users can also choose to download Fiji at https://fiji.sc/. After downloading, unpack it and use it directly. Fiji is an image processing package – a "batteries-included" distribution of ImageJ, bundling a lot of plugins which facilitate scientific image analysis.

We recommend that users download the latest version of ImageJ or Fiji.

2.2 How to install DecodeSTORM

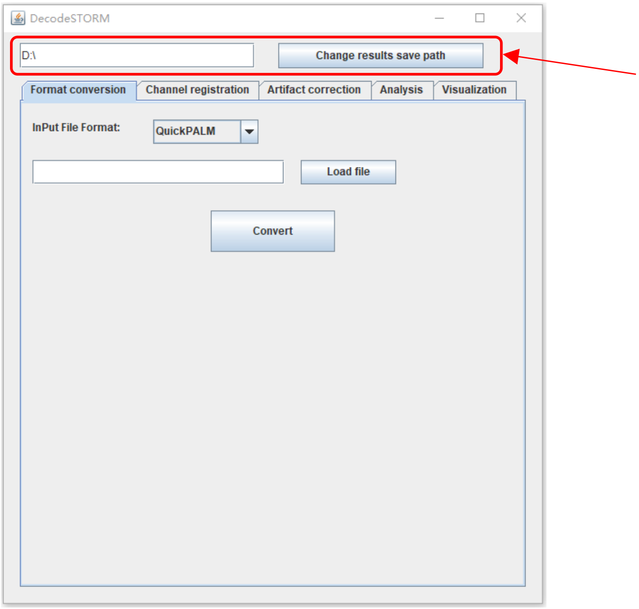
DecodeSTORM is built for ImageJ independently. To install, simply copy four dynamic link libraries (cudart64_110.dll, libopenblas.dll, opencv_world450.dll, DecodeSTORM_CPPDLL.dll) files into the ImageJ installation folder. And then copy the corresponding .jar plug-in file to the plugins folder of ImageJ.

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 path to save the results of format conversion, artifact correction, quantitative analysis, and visualization. The default path is D:\.

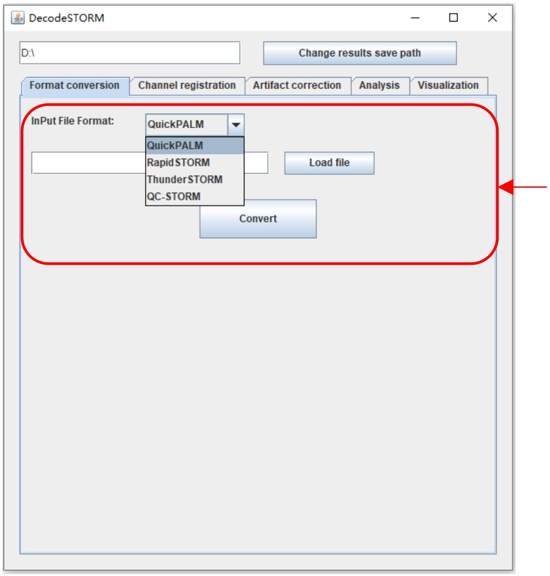


Change the path to save the results of format conversion, artifact correction, and quantitative analysis.

Figure 1: Change the path to save results.

3.2 Format conversion

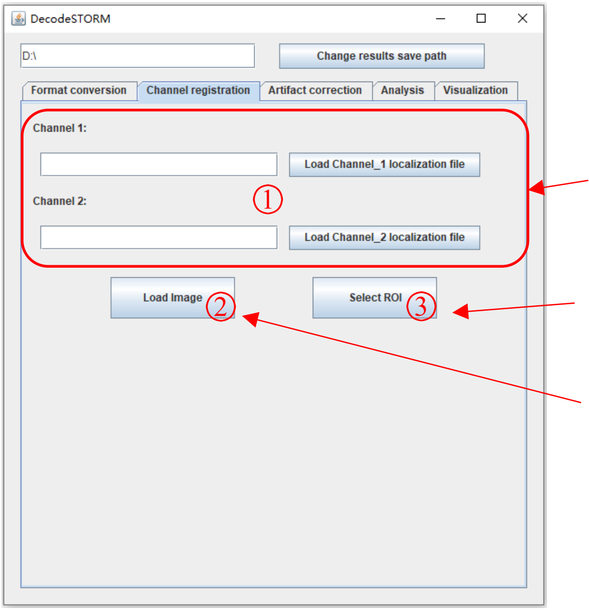
Format conversion module is used to convert the localization table generated by QuickPALM, RapidSTORM, ThunderSTORM, or QC-STORM into the format suitable for DecodeSTORM. First, select the file format that users need to convert, then click the "Load file" button to upload the localization table, and finally click the "Convert" button. Results are saved at the selected path.



Format convert to get the localization table suitable for DecodeSTORM.

Figure 2: Format conversion module.

3.3 Channel registration



D:\

Load the localization table.

Select ROI to artifact correction and/or quantitative analysis.

Load data scatter image.

Figure 3: Channel registration module.

Load localization file: DecodeSTORM provides two channels for importing two-color SMLM data: channel 1 for green and channel 2 for red. For single-color SMLM data, users can click the “Load Channel_1 localization file” or “Load Channel_2 localization file” button to upload. For two-color SMLM data, users should click the “Load Channel_1 localization file” and “Load Channel_2 localization file” buttons to upload.

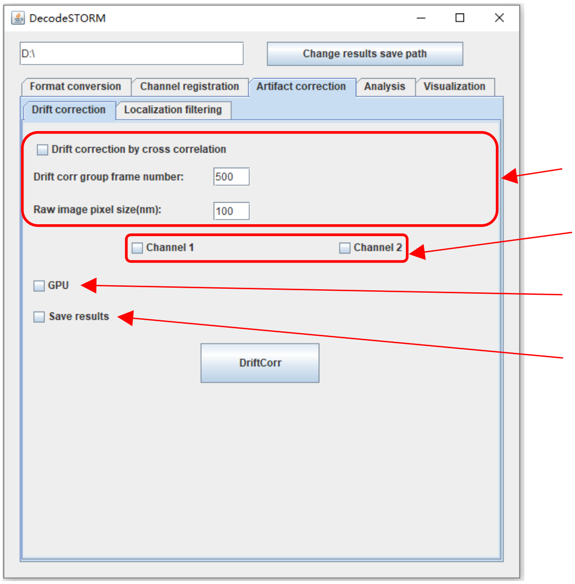
Load image: Clicking the "Load Image" button will generate a scatter image of the data when the localization table is in the channel.

Select ROI: Select ROI for artifact correction and quantitative analysis. First, Click the ImageJ “Rectangle” button to select the ROI area. Then, 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

DecodeSTORM provides redundant cross-correlation drift correction to correct artifacts caused by sample drift. A single frame contains too few localizations for reliable correlation calculation, so users need to set the number of drift correction group frames (default: 500). In addition, users need to set the original image pixel size (default: 100), then select one or all channels and click on the “DrifCorr” button for drift correction, the results can be selected to be saved or not. 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).



D:\

□ Drift correction by cross correlation

Drift corr group frame number: 500

Raw image pixel size(nm): 100

□ Channel 1 □ Channel 2

□ GPU

□ Save results

Lateral drift correction.

Select channel to drift correction.

Select GPU.

Select save results.

Figure 4: Drift correction in Artifact correction module.

3.4.2 Localization filtering

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

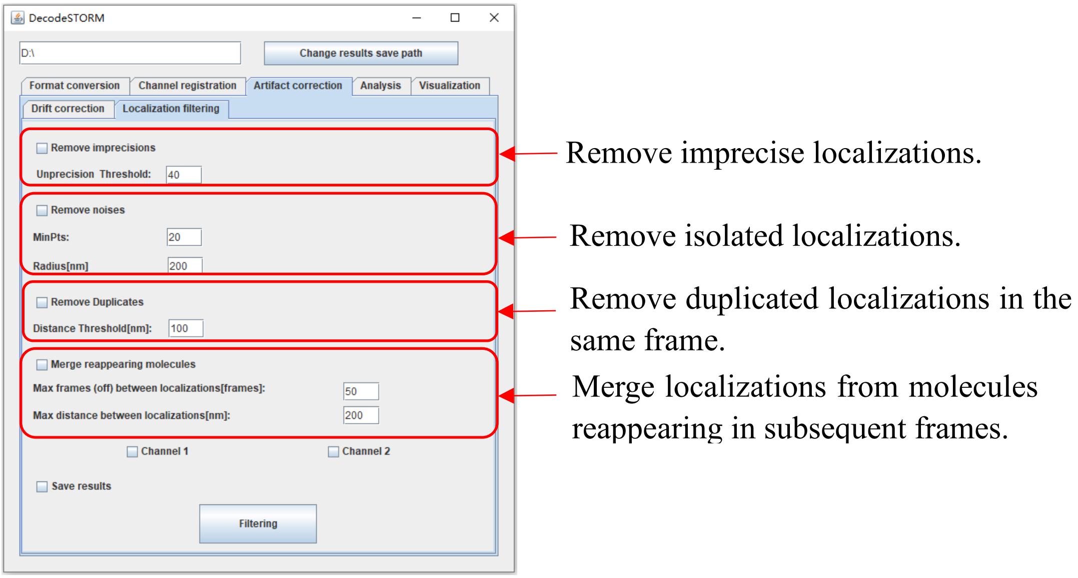
Remove imprecise localizations: This function requires localization tables to have localization precision or to be calculated using other information. To remove imprecise localizations, users need to set an imprecise localization threshold (default: 40).

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 (default: 200nm) and the minimum number of localization events within the radius (default: 20).

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 (default: 100nm) to remove duplicated localizations.

Merge localizations from molecules reappearing in subsequent frames: Localizations from molecules reappearing in subsequent frames are determined according to the euclidean distance between localizations in the subsequent frame. In DecodeSTORM, users need to set 2 parameters to merge localizations from molecules reappearing in subsequent frames: max frames of localizations reappearing (default: 50) and max distance between localizations (default: 200nm).

After the user selects the method, clicking the "Filtering" button will generate the filtered scatter plot. If users choose to save the results, the results users get using either method will be saved.



D:\

□ Remove imprecisions
Unprecision Threshold: 40

□ Remove noises
MinPts: 20
Radius[nm] 200

□ Remove Duplicates
Distance Threshold[nm]: 100

□ Merge reappearing molecules
Max frames (off) between localizations[frames]: 50
Max distance between localizations[nm]: 200

□ Channel 1 □ Channel 2

□ Save results

Remove imprecise localizations.

Remove isolated localizations.

Remove duplicated localizations in the same frame.

Merge localizations from molecules reappearing in subsequent frames.

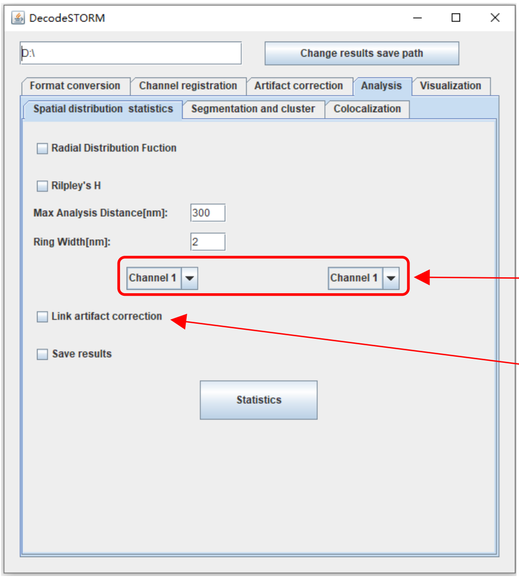
Figure 5: Localization filtering in Artifact correction module.

3.5 Quantitative analysis

3.5.1 Spatial distribution statistics

In the spatial distribution statistics, the max analysis distance (default: 300) and ring width (default: 2nm) 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' data. Radial Distribution Function (*G(r)*) and Ripley's

H Function (*H(r)*) can be used separately or together. Clicking the "Statistics" button will generate their function graphs. If users choose to save the result, not only the function graph is saved, but also the function value and its corresponding radius r are saved in a CSV file. 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”.



DecodeSTORM

D:\ | Change results save path

Format conversion | Channel registration | Artifact correction | Analysis | Visualization

Spatial distribution statistics | Segmentation and cluster | Colocalization

□ Radial Distribution Fuction

□ Rilpley's H

Max Analysis Distance[nm]: 300

Ring Width[nm]: 2

Channel 1 ▾ Channel 1 ▾

□ Link artifact correction

□ Save results

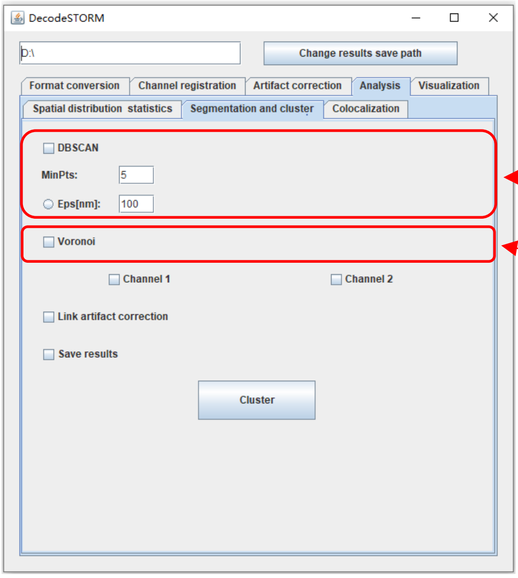
Statistics

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

Select link artifact correction.

Figure 6: Spatial distribution statistics in Quantitative analysis module.

3.5.2 Segmentation and cluster



DecodeSTORM

D:\ | Change results save path

Format conversion | Channel registration | Artifact correction | Analysis | Visualization

Spatial distribution statistics | Segmentation and cluster | Colocalization

□ DBSCAN

MinPts: 5

○ Eps[nm]: 100

□ Voronoi

□ Channel 1 □ Channel 2

□ Link artifact correction

□ Save results

Cluster

Density-based spatial clustering of applications with noise.

Voronoi Tessellation

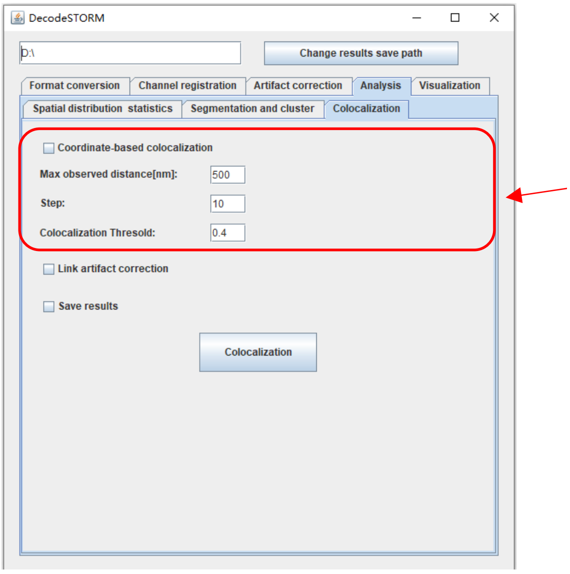
Figure 7: Segmentation and cluster in Quantitative analysis module.

DBSCAN has two parameters: the radius *eps* (default 100nm) that determines the size of the search nearest neighbor localization event region and the minimum number of localization events *MinPts* (default 5) in the above region, which generates the molecular segmentation and cluster maps and gets the number of clusters. In DecodeSTORM, *eps* can also be calculated automatically. Voronoi can cluster localizations to generate a Voronoi diagram without setting any parameters.

DBSCAN and Voronoi can be used separately or together. Clicking the "Cluster" button will generate a DBSCAN clustering map and/or Voronoi diagram. If users choose to save the results, the DBSCAN clustering map and/or Voronoi diagram will be saved, in addition, the specific clustering information of DBSCAN will be saved in a CSV file.

3.5.3 Colocalization

In DecodeSTORM, colocalization analysis is performed with three parameters to be set: maximum observation distance $R_{\max}$, search step *Step* and CBC threshold. The CBC threshold allows the user to autonomously segment colocalization and uncolocalization localizations. Clicking the "Colocalization" button will generate histograms of CH1 → CH2 and CH2 → CH1 colocalization values. If users choose to save the result, the above histogram and a CSV file are saved, the CSV file contains the percentage of the number of localizations whose colocalization value of CH1 → CH2 is greater than the threshold to the number of localizations in CH1 and the percentage of the number of localizations whose colocalization value of CH2 → CH1 is greater than the threshold to the number of localizations in CH2.



D:\

□ Coordinate-based colocalization

Max observed distance[nm]: 500

Step: 10

Colocalization Thresold: 0.4

□ Link artifact correction

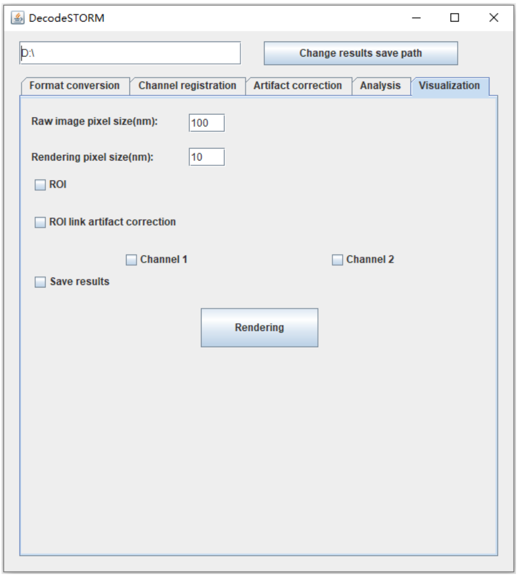
□ Save results

Coordinate-based colocalization

Figure 8: Colocalization in Quantitative analysis module.

3.6 Visualization

In addition to the generation of the scattered map, DecodeSTORM provides Gaussian rendering for SMLM data visualization, and users need to set the rendering pixel sizes, and raw image pixel size, and then click the "Rendering" button will generate a rendering image. We also provide an ROI option where users can render the selected ROI data.



D:\

Raw image pixel size(nm): 100

Rendering pixel size(nm): 10

□ ROI

□ ROI link artifact correction

□ Channel 1 □ Channel 2

□ Save results

Figure 9: Visualization module.

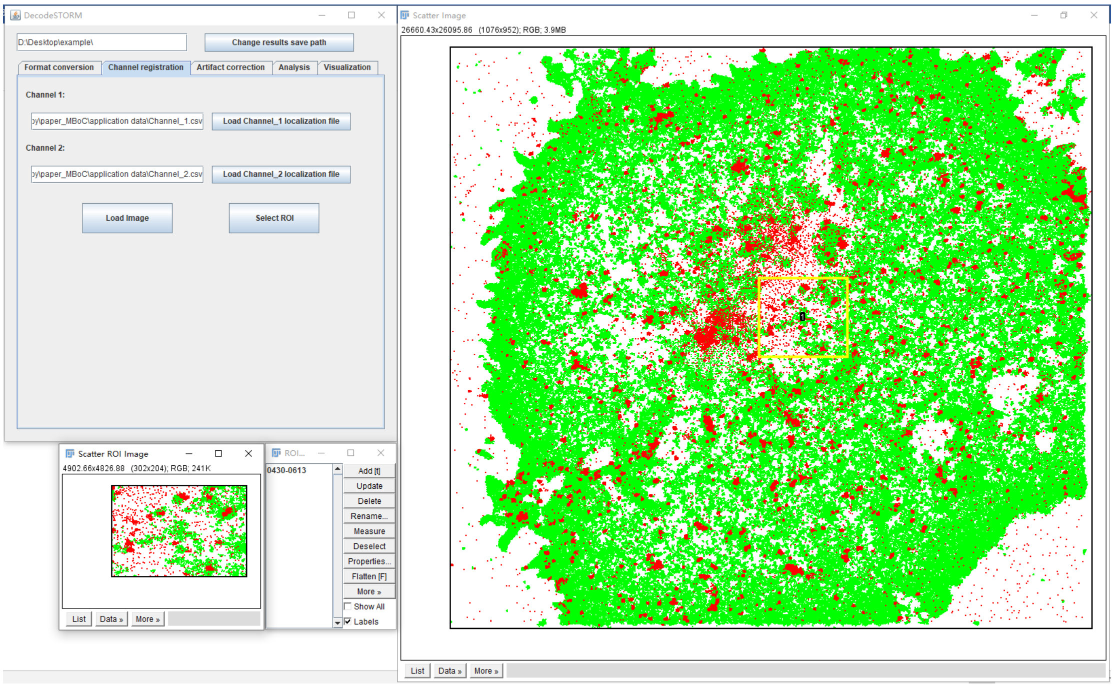
4. Note

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. Testing of DecodeSTORM with experimental data

We used two-color data for the test, which were provided by Pageon et al. in their Github repository (https://github.com/PRNicovich/ClusDoC) when developing Clus-DOC. The two-color SMLM data were imaged from TCR (green) and pTCR (red), respectively, in activated Jurkat cells. The ROI we selected was also saved, and after importing the image, the saved ROI is loaded for testing.

Load localization file → Load Image → Click on the top right corner to enlarge the button → ”Ctrl + T” → ROI Manager → More → Open → Open 0430-0613.roi → Click on the selected → Select ROI. The specific operation results are shown in the following figures.



D:\Desktop\example\

Channel 1: ɔy\paper_MBoC\application data\Channel_1.csv

Channel 2: ɔy\paper_MBoC\application data\Channel_2.csv

4902.66x4826.88 (302x204); RGB; 241K

0430-0613

26660.43x26095.86 (1076x952); RGB; 3.9MB

Figure 10: Upload data and select ROI.

5.1 Artifact correction

5.1.1 Drift correction

This data lacks the information needed for the redundant inter-correlation drift correction method, so we did not perform a drift correction.

5.1.2 Localization filtering

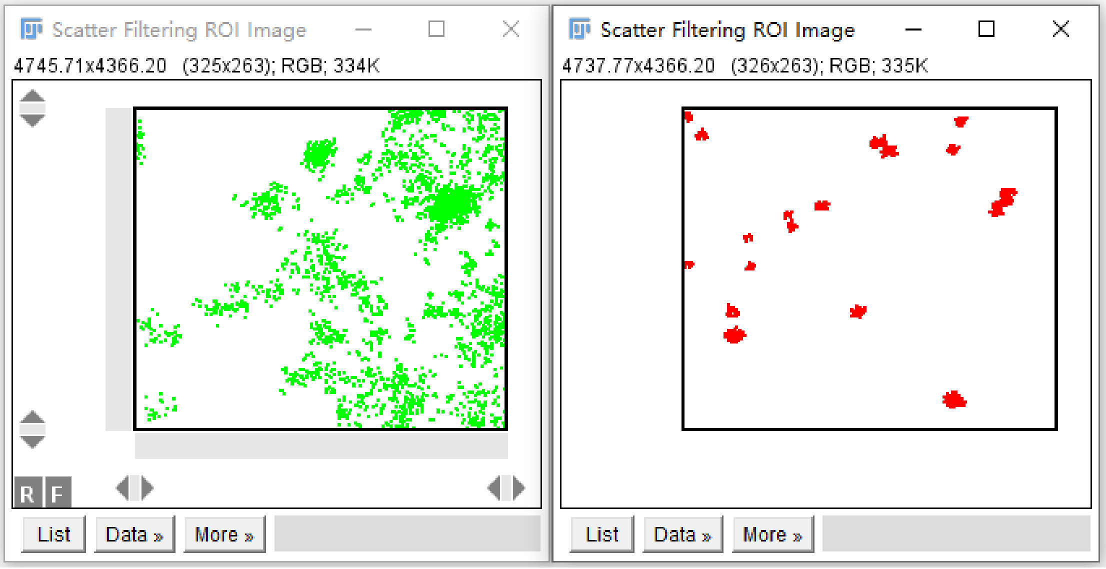

Scatter Filtering ROI Image
4745.71x4366.20 (325x263); RGB; 334K
R F
List
Data »
More »
Scatter Filtering ROI Image
4737.77x4366.20 (326x263); RGB; 335K
List
Data »
More »

Figure 11: Scatter map of filtered data within channel 1 and channel 2.

5.2 Quantitative analysis of raw data

5.2.1 Spatial Statistics: *G(r)* and *H(r)*

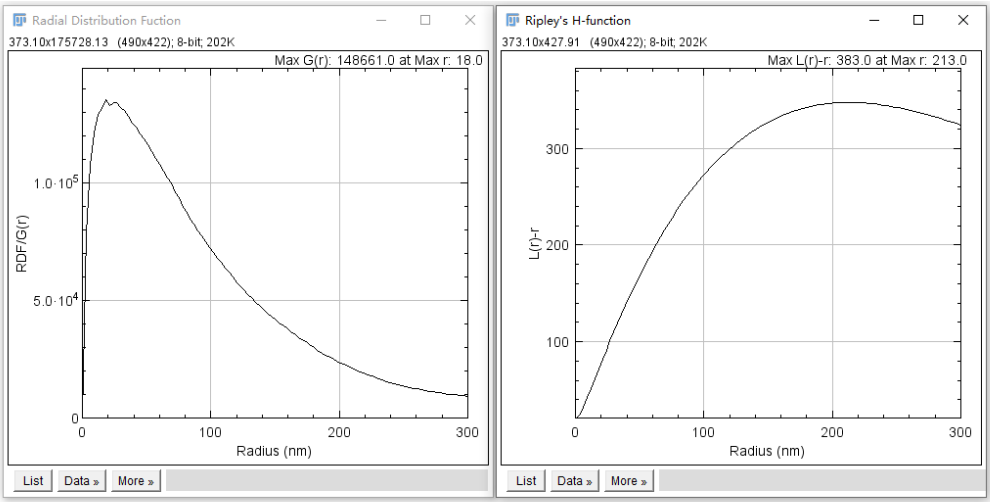


Figure 12: *G(r)* and *H(r)* of raw channel 1 data.

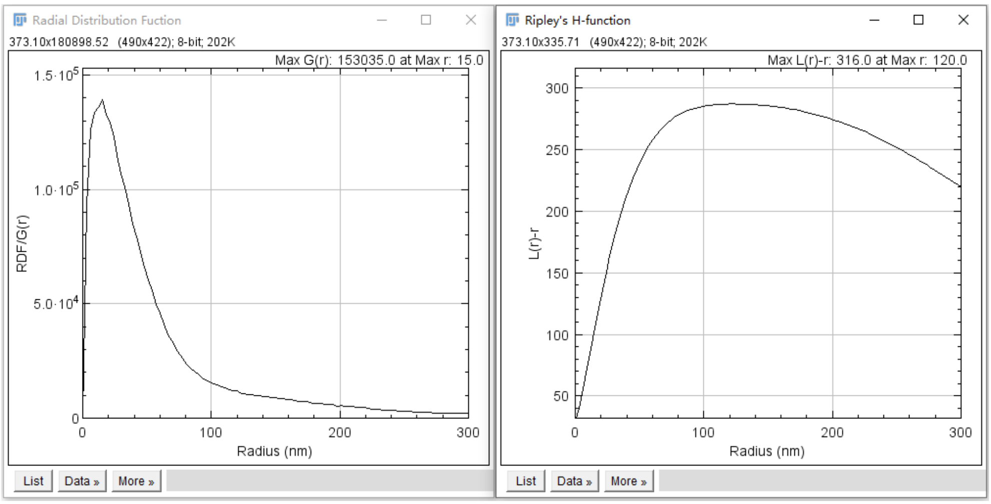
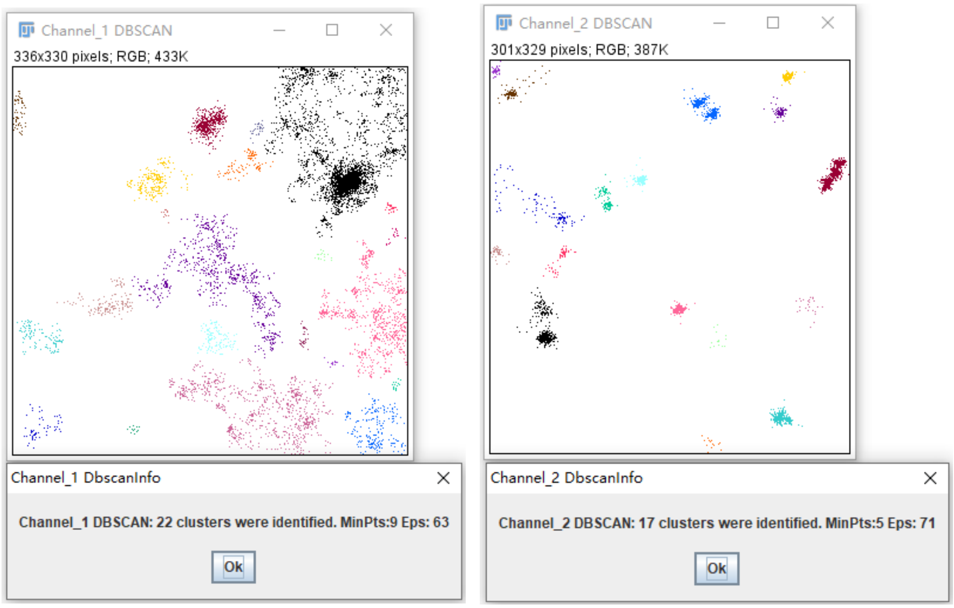


Figure 13: *G(r)* and *H(r)* of raw channel 2 data.

5.2.2 Segmentation and cluster: DBSCAN and Voronoi



Channel_1 DBSCAN: 22 clusters were identified. MinPts:9 Eps: 63

Channel_2 DBSCAN: 17 clusters were identified. MinPts:5 Eps: 71

Figure 14: DBSCAN clustering maps of raw channel 1 and channel 2 data.

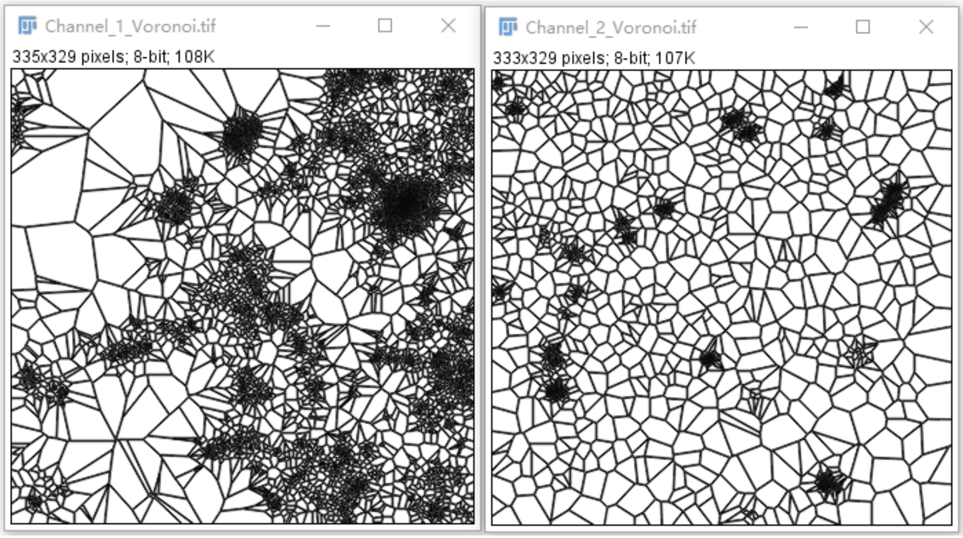


Figure 15: Voronoi diagram of raw channel 1 and channel 2 data.

5.2.3 Colocalization

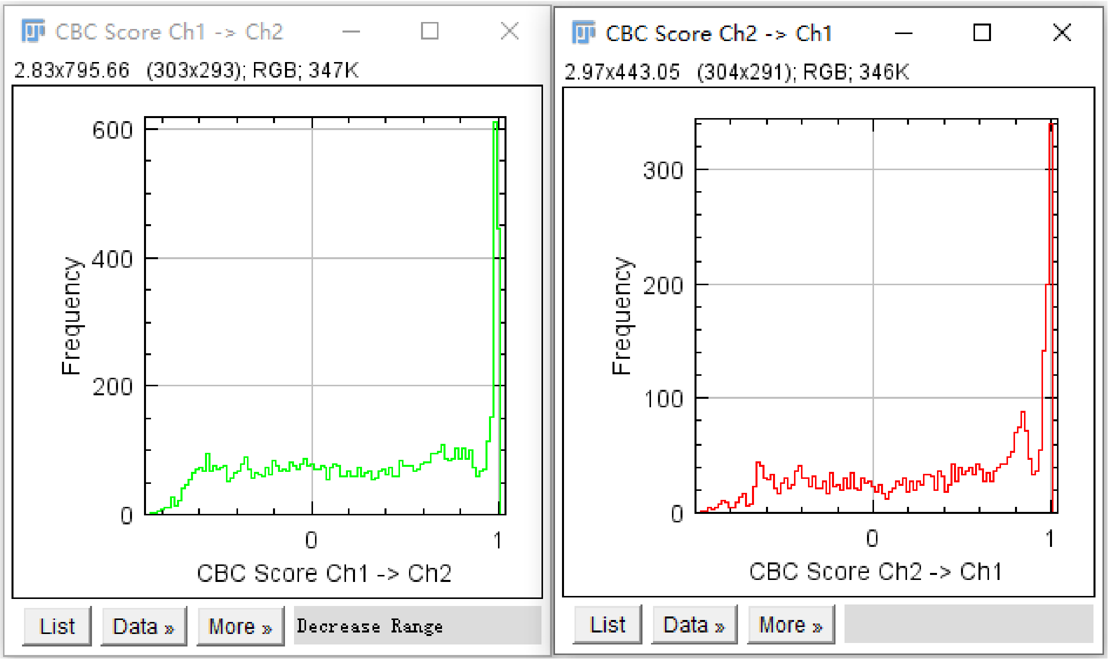


Figure 16: The histograms of CH1 → CH2 and CH2 → CH1 colocalization value without artifact correction.

5.3 Quantitative analysis of artifact correction data

5.3.1 Spatial Statistics: *G(r)* and *H(r)*

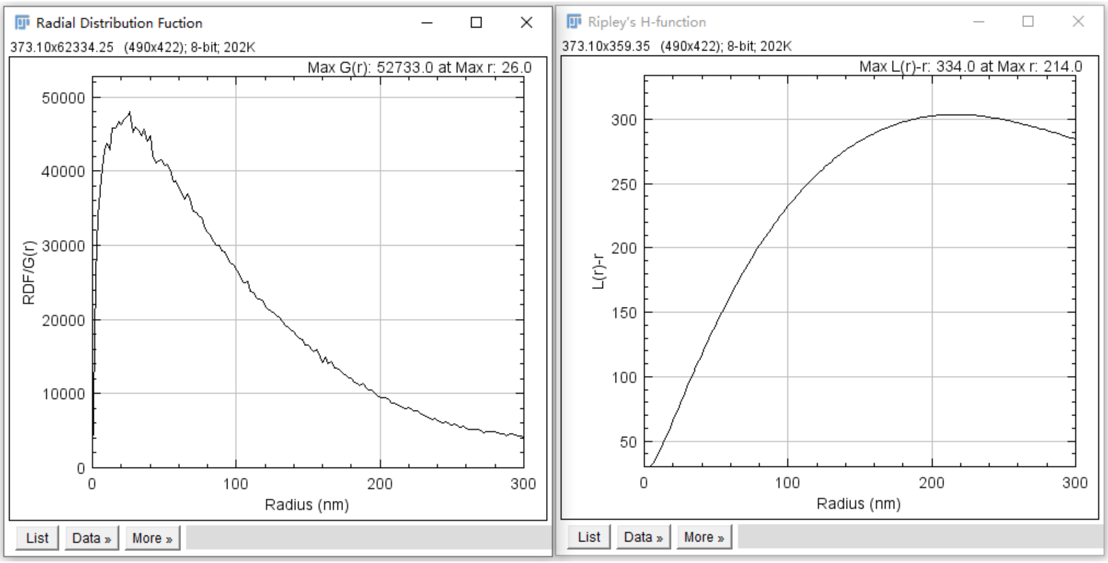


Figure 17: *G(r)* and *H(r)* of artifact-corrected channel 1 data.

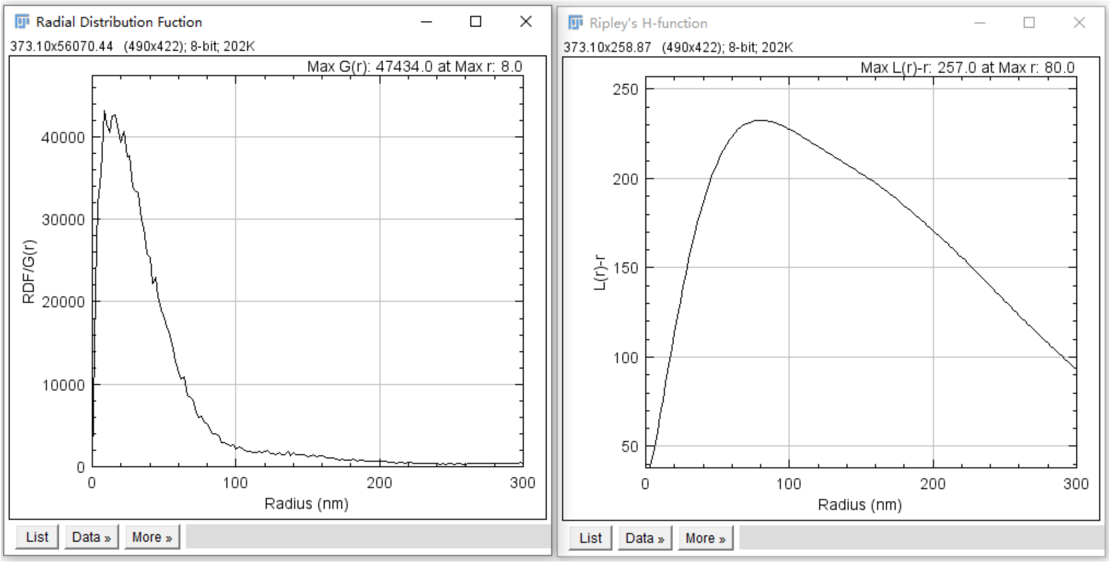
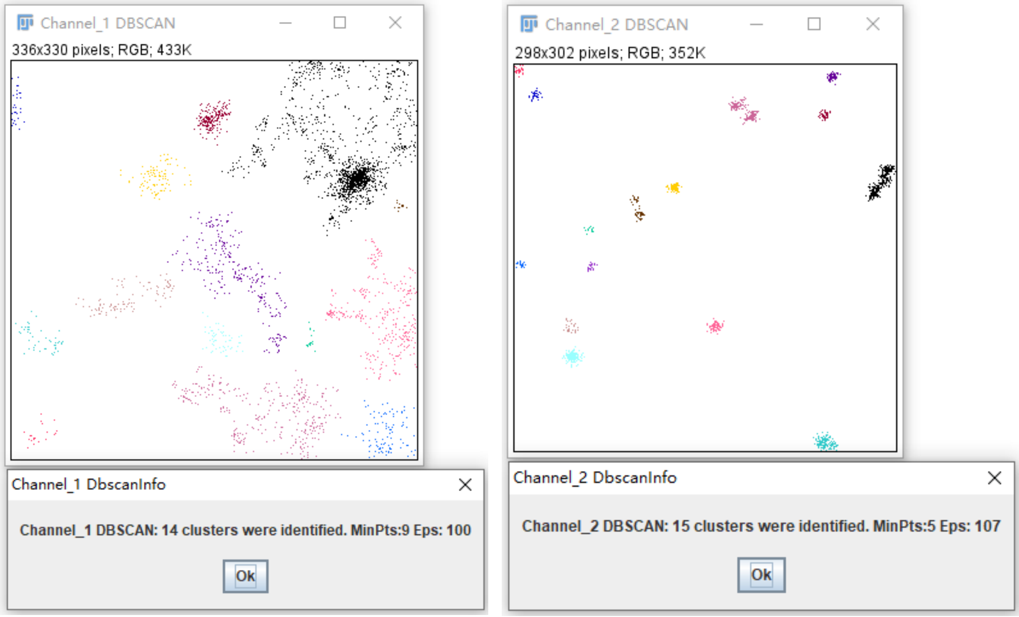


Figure 18: *G(r)* and *H(r)* of artifact-corrected channel 2 data.

5.3.2 Segmentation and cluster: DBSCAN and Voronoi



Channel_1 DBSCAN: 14 clusters were identified. MinPts:9 Eps: 100

Channel_2 DBSCAN: 15 clusters were identified. MinPts:5 Eps: 107

Figure 19: DBSCAN clustering maps of artifact-corrected channel 1 and channel2 data.

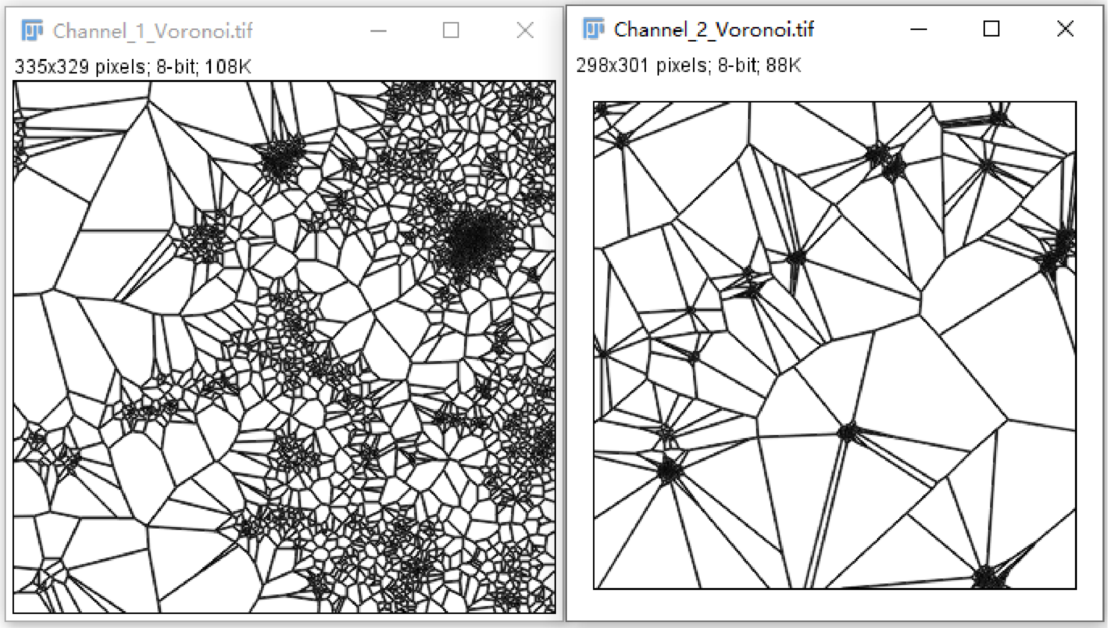


Figure 20: Voronoi diagram of artifact-corrected channel 1 and channel2 data.

5.3.3 Colocalization

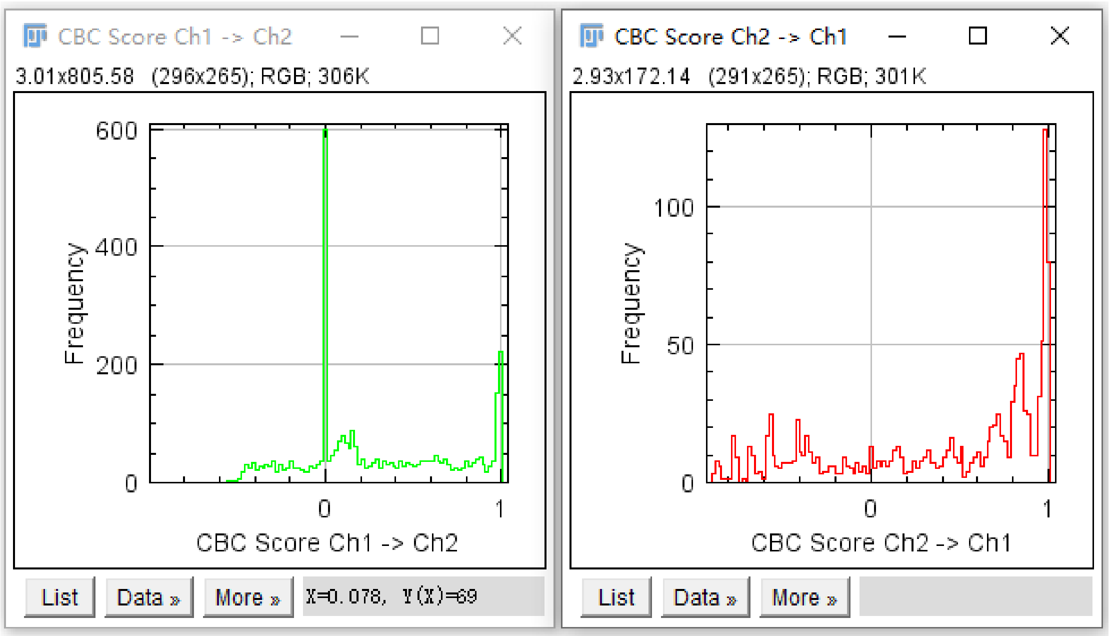


Figure 21: The histograms of CH1 → CH2 and CH2 → CH1 colocalization value with artifact correction.