User's guide of DecodeSTORM

February 20, 2022

Contents

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 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).

2.2 How to install DecodeSTORM

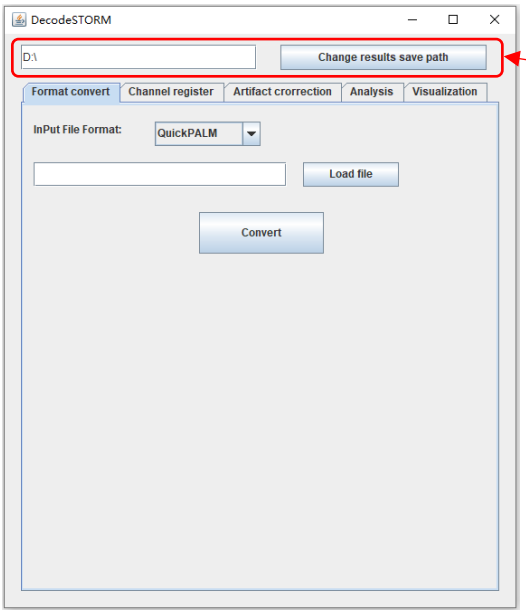
DecodeSTORM is built for ImageJ independently. To install, simply copy four dynamic link library (cudart64_110.dll, libopenblas.dll, opencv_world450.dll, DecodeSTORM_CPPDLL.dll) files into the ImageJ installation folder. And then copy the corresponding .jar plug-in file to the plugins folder of ImageJ.

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

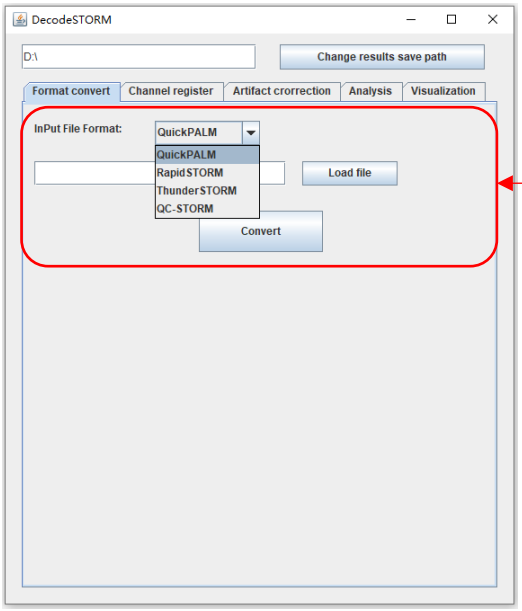


Change the save path of format convert, artifact correction and quantitative analysis results

Figure S1: Change results save path.

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. First select the file format 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 S2: Format convert.

3.3 Channel register

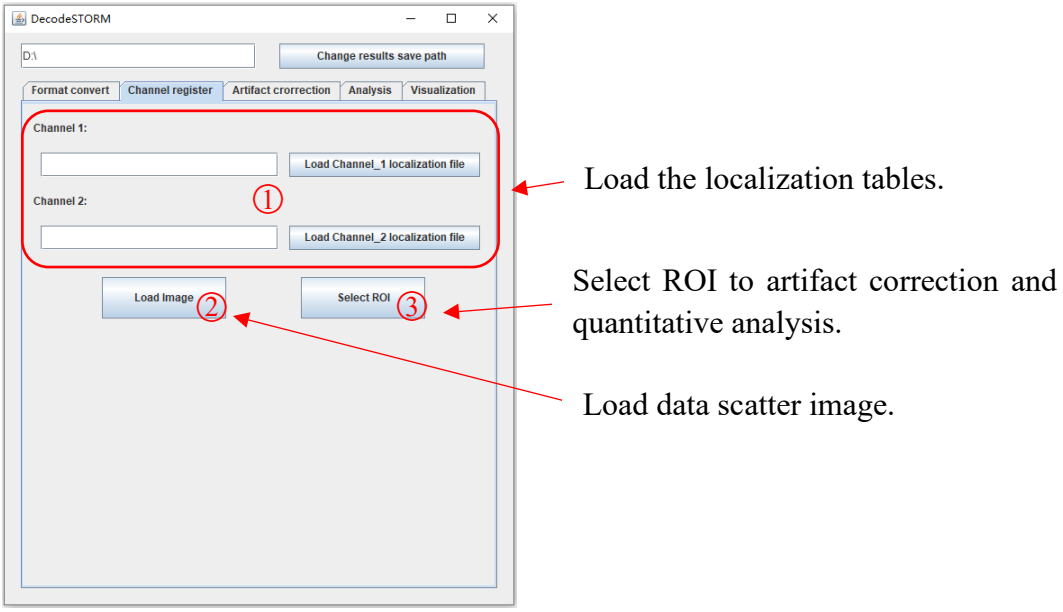


Figure S3: Channel register.

Load localization file: DecodeSTORM has double color channels: channel 1 (green) and channel 2 (red). A single localization table can be click the “Load Channel_1 localization file” or “Load Channel_2 localization file” button to upload. Double localization table (double color data) should be click the “Load Channel_1 localization file” and “Load Channel_2 localization file” button 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, 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

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 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).

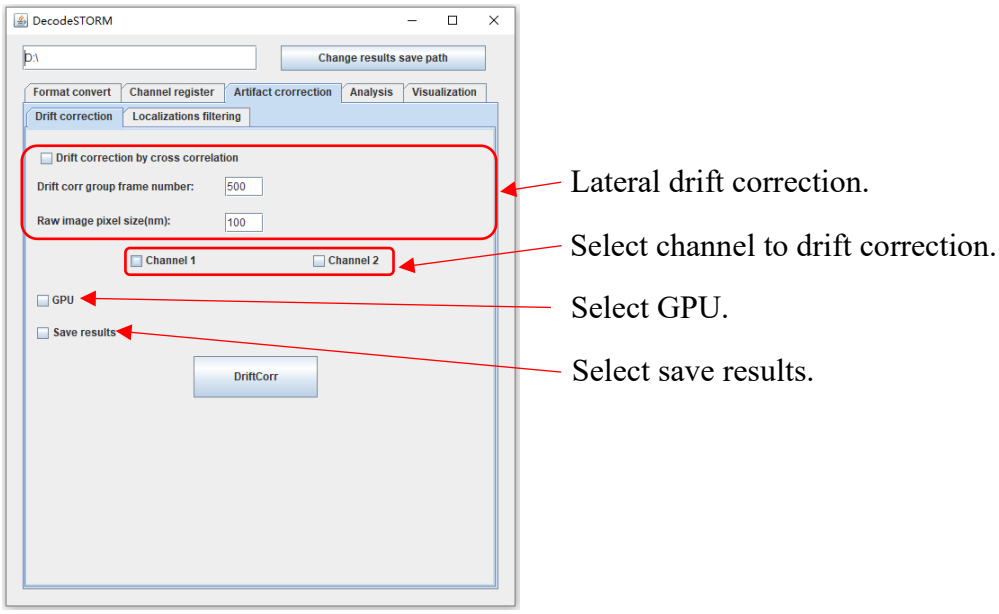


Figure S3: Drift correction.

3.4.2 Localizations filter

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 (default: 40) 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 (default: 200nm) and the standard deviation to determine 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 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 (default: 50) and max distance between localizations (default: 200nm).

After the users 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.

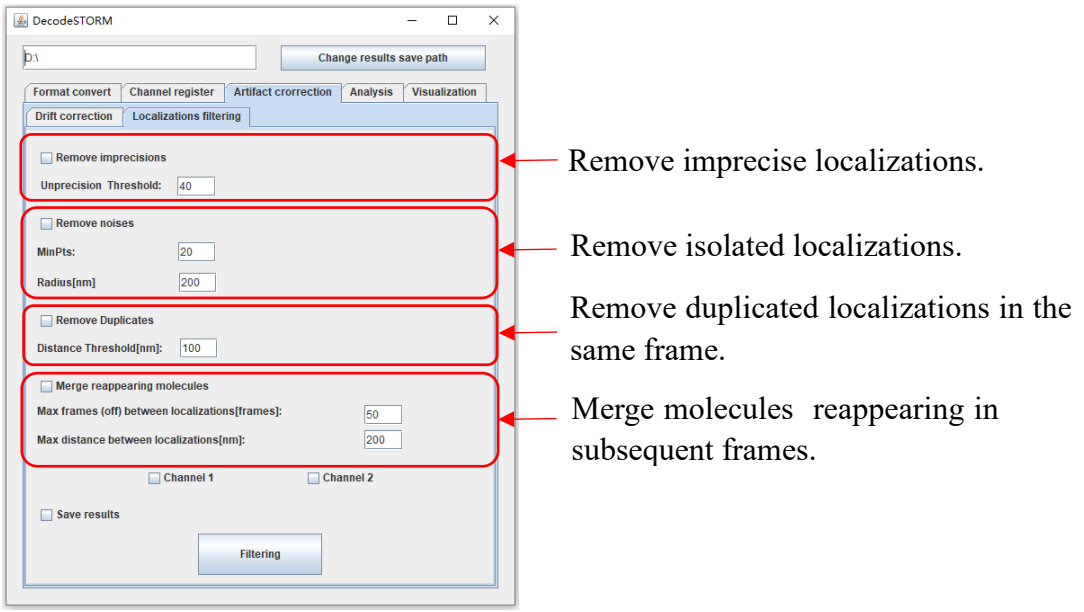


Figure S5: Localizations filter.

3.5 Quantitative analysis

3.5.1 Spatial distribution statistics

In the spatial distribution statistics module, 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. Radial Distribution Function (RDF) and Ripley's H Function 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".

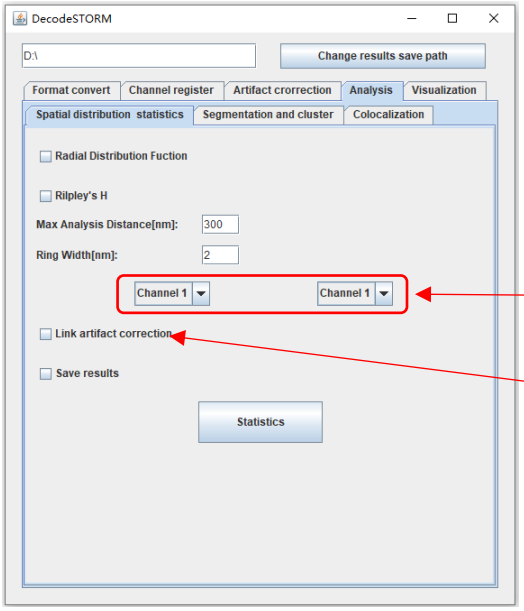


Figure S6: Spatial distribution statistics.

3.5.2 Segmentation and cluster

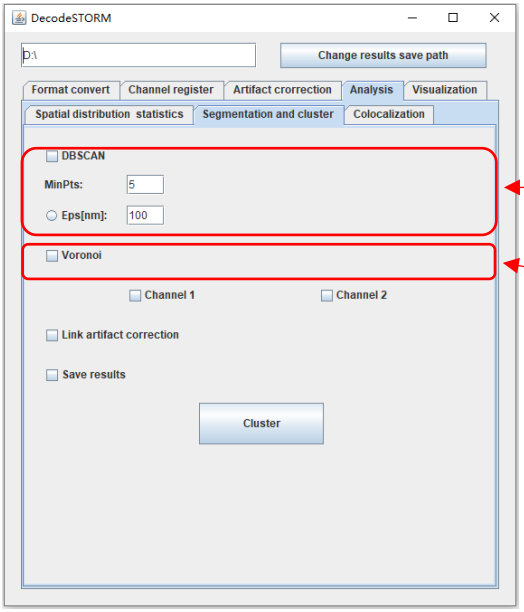


Figure S7: Segmentation and cluster.

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 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 disgram without setting any parameters.

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

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 value. 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 molecules whose colocalization value of CH1 → CH2 is greater than the threshold to the number of molecules in CH1 and the percentage of the number of molecules whose colocalization value of CH2 → CH1 is greater than the threshold to the number of molecules in CH2.

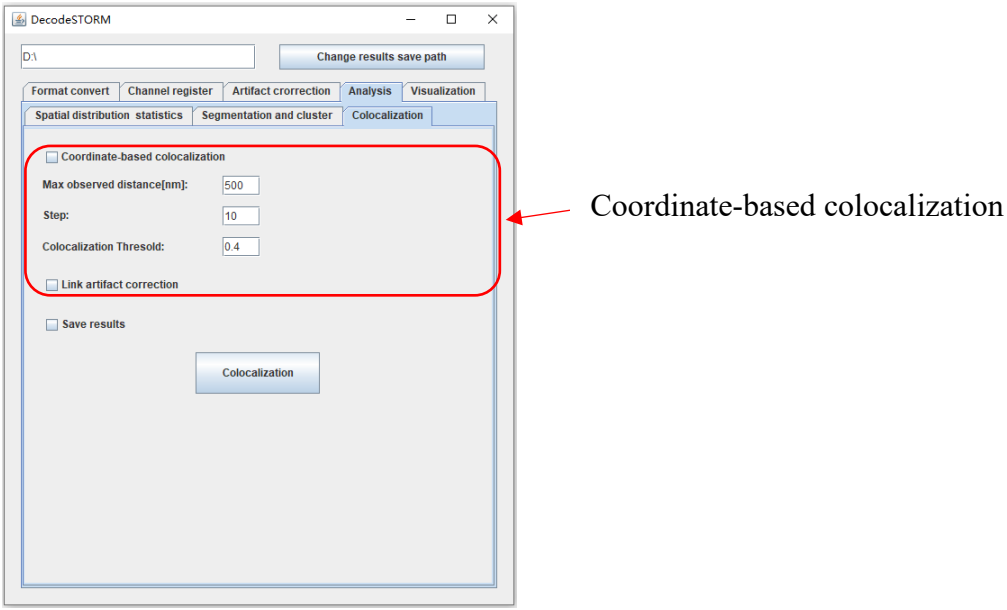


Figure S8: Colocalizaiton.

3.6 Visualization

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

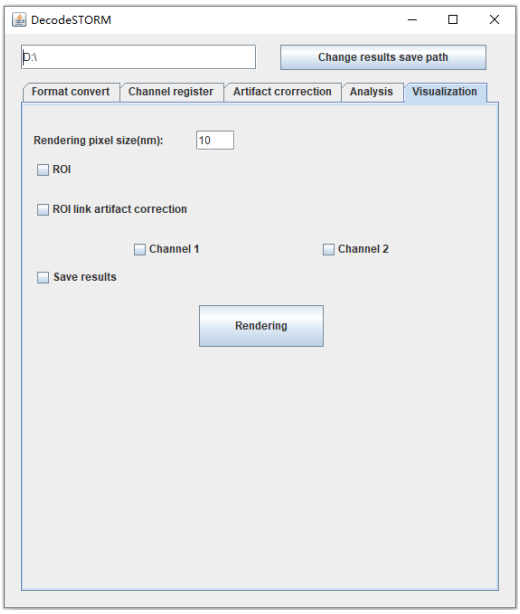


Figure S9: Visualizaition.

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. Application of DecodeSTORM in experimental data

We used two-color data for the application, which were provided by Pageon et al. in their Github repository when developing Clus-DOC SMLM data of T-cell receptor (TCR; green) and phosphorylated TCR (pTCR; red) in activated Jurkat cells (https://github.com/PRNicovich/ClusDoC). 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.

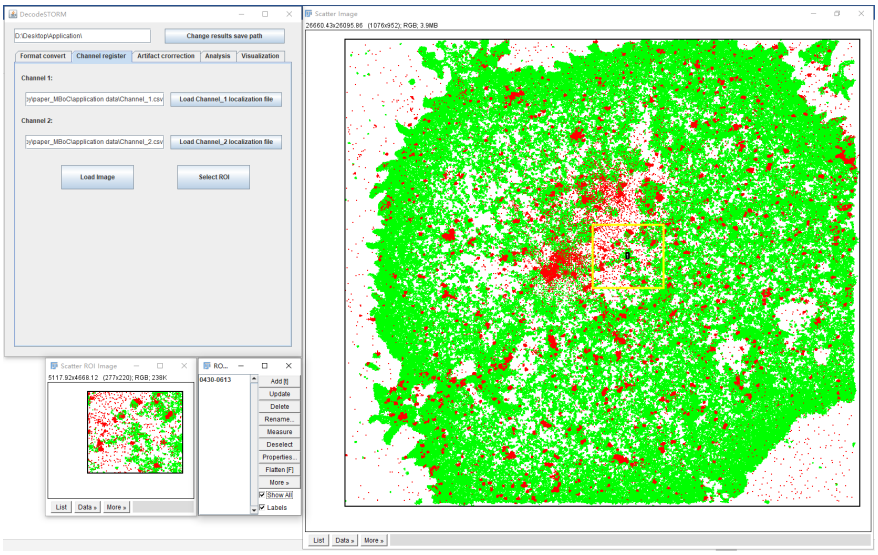


Figure S9: Upload data and select ROI.

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 Localizations filtering

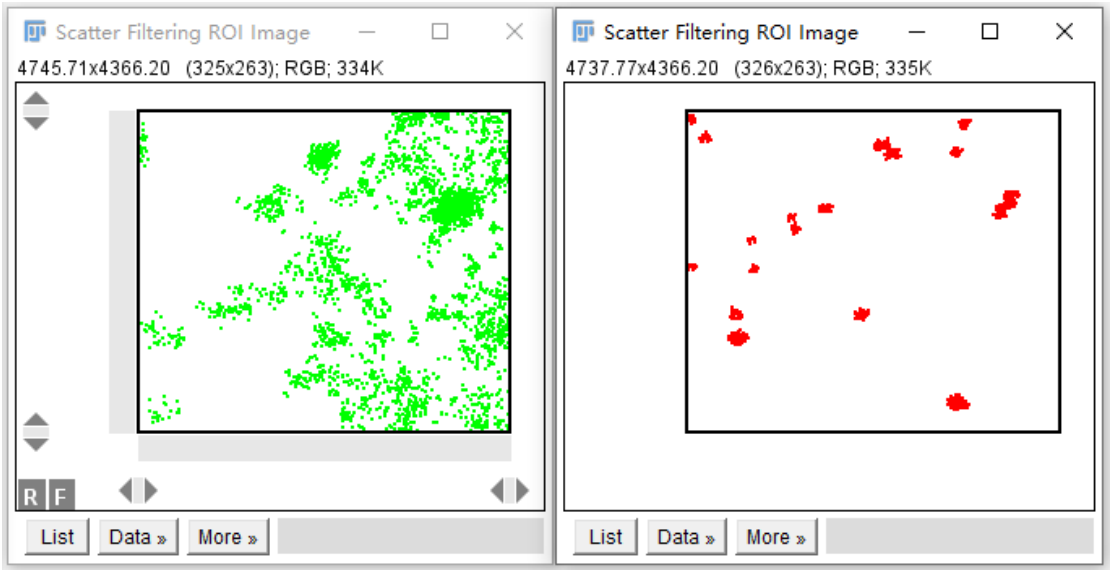


Figure S10: Scatter plot of filtered data within channel 1 and channel2.

5.2 Quantitative analysis of raw data

5.2.1 Spatial Statistics: RDF and Ripley's H Function

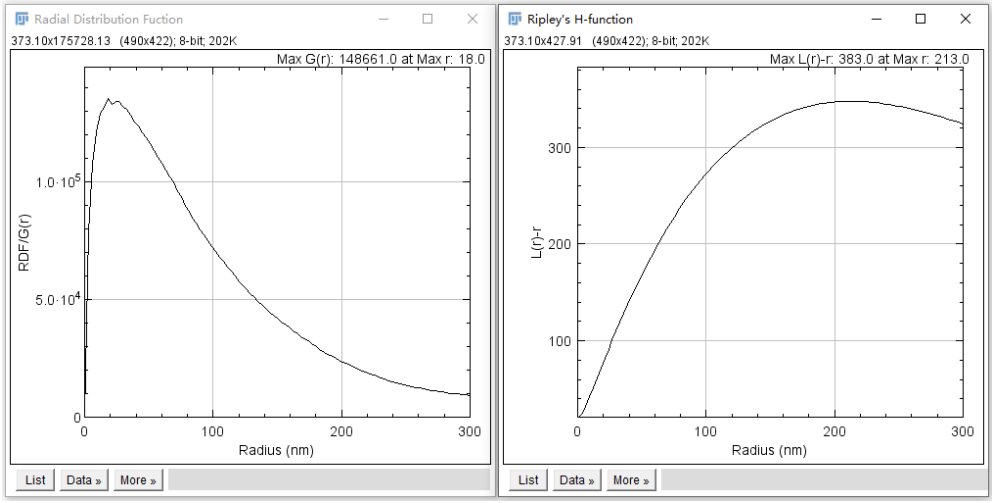


Figure S11: G(r) and H(r) of channel 1.

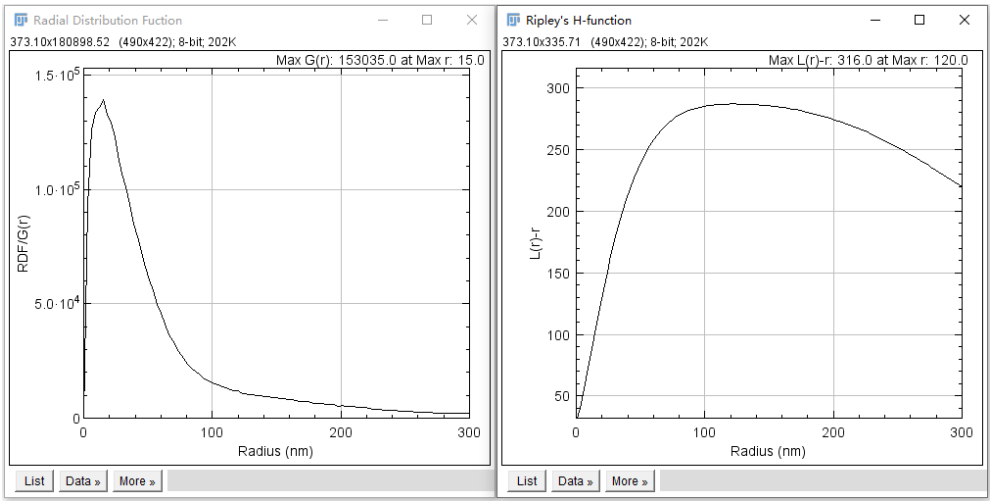


Figure S11: G(r) and H(r) of channel 2.

5.2.2 Segmentation and cluster: DBSCAN and Voronoi

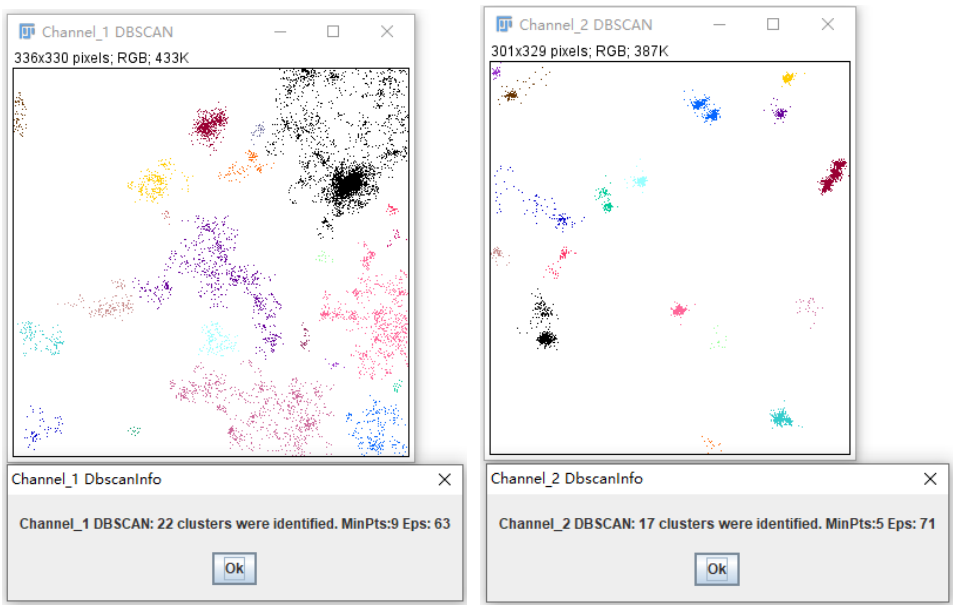


Figure S12: DBSCAN clustering maps of channel 1 and channel2.

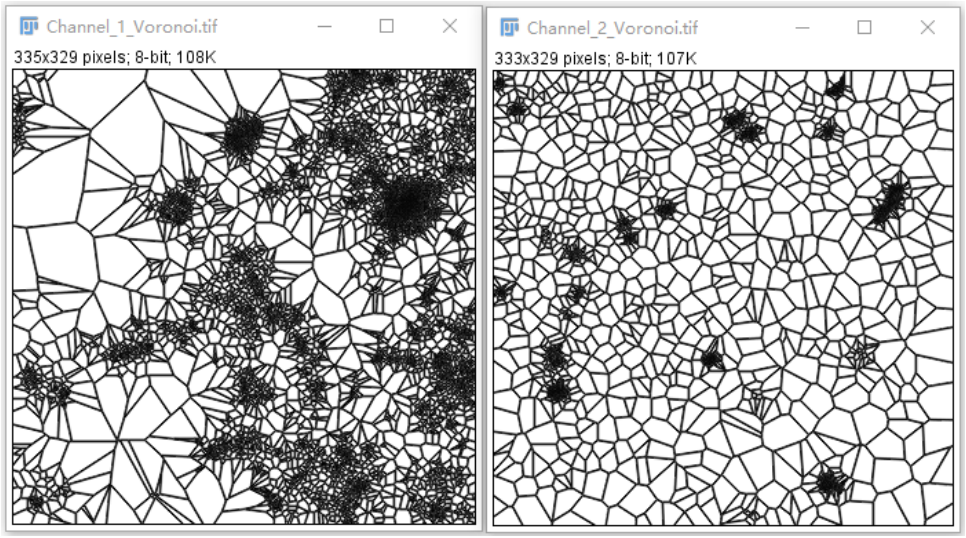


Figure S13: Voronoi disgram of channel 1 and channel2.

5.2.3 Colocalization

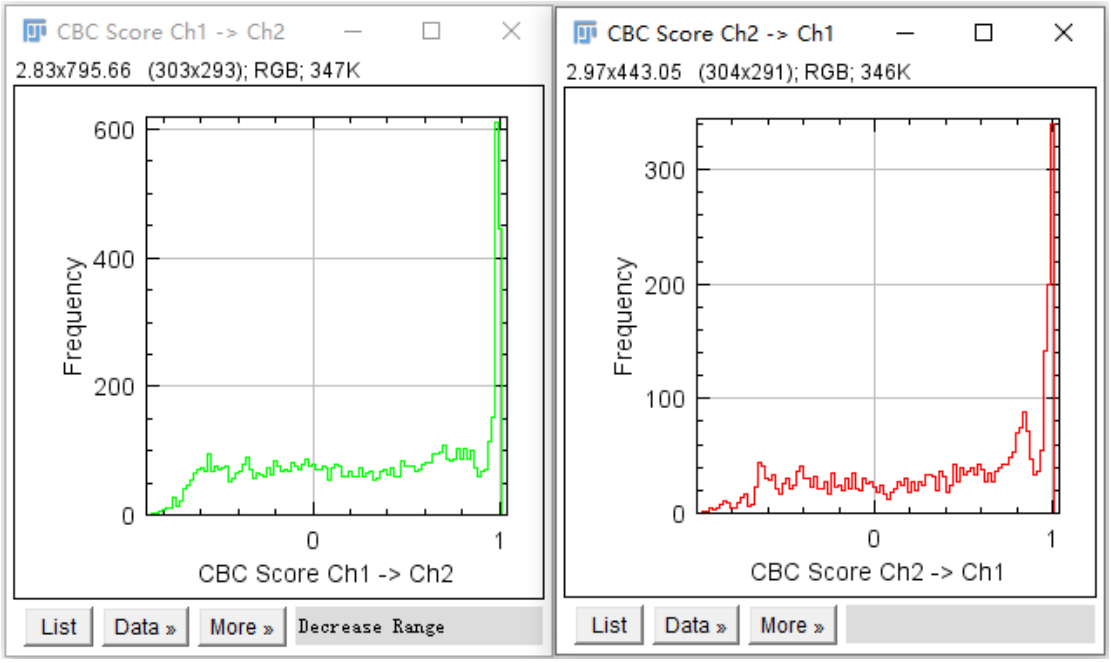


Figure S14: The histograms of CH1 → CH2 and CH2 → CH1 colocalization value.

5.3 Quantitative analysis of artifact correction data

5.3.1 Spatial Statistics: RDF and Ripley's H Function

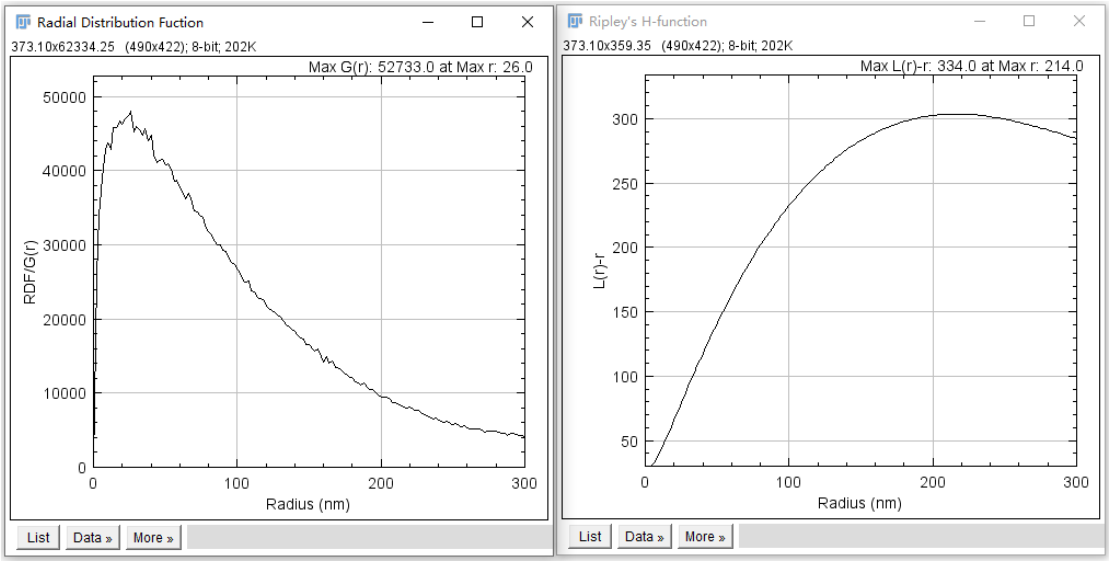


Figure S15: G(r) and H(r) of channel 1 with artifact correction.

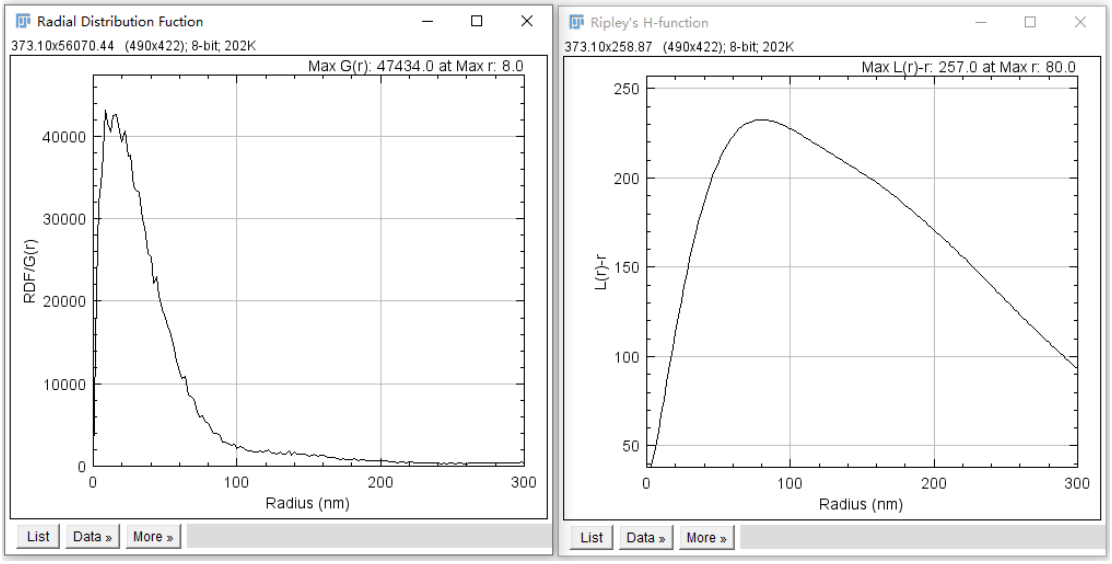


Figure S16: G(r) and H(r) of channel 2 with artifact correction.

5.3.2 Segmentation and cluster: DBSCAN and Voronoi

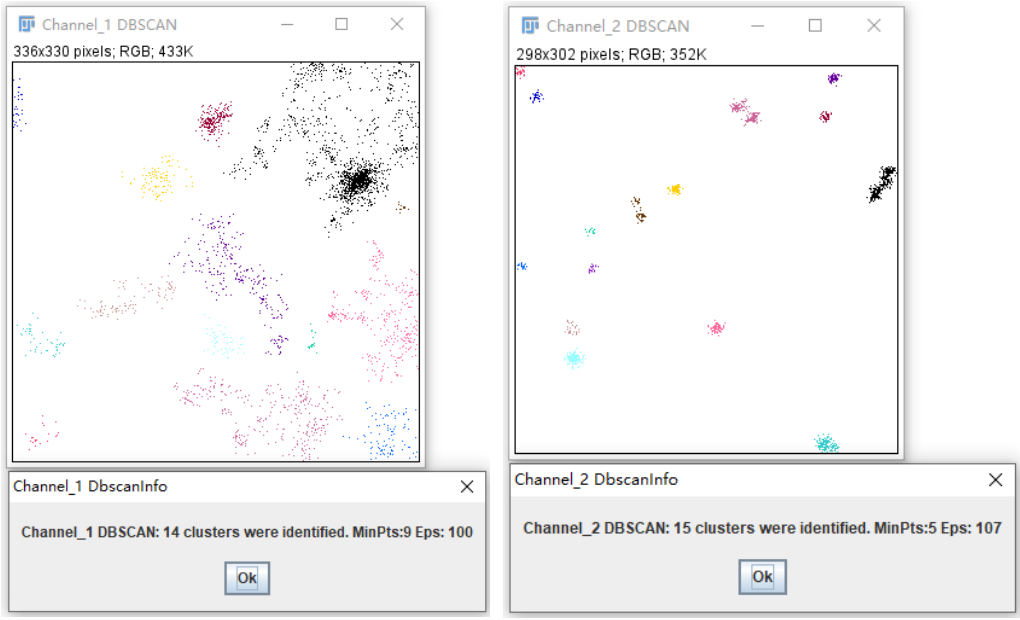


Figure S17: DBSCAN clustering maps of channel 1 and channel2 with artifact correction.

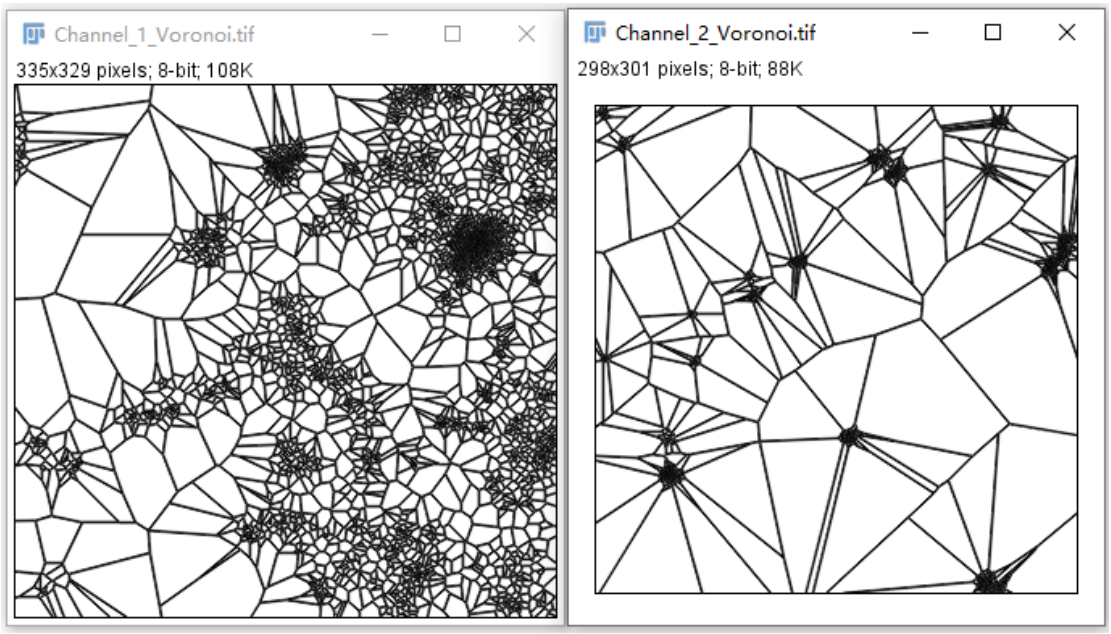


Figure S18: Voronoi disgram of channel 1 and channel2 with artifact correction.

5.3.3 Colocalization

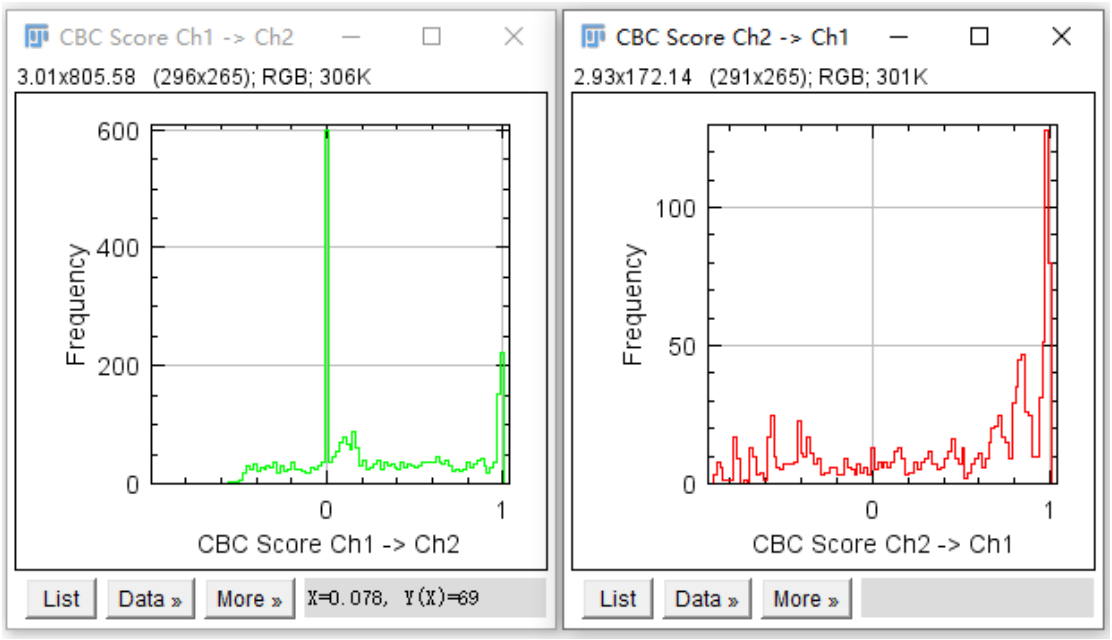


Figure S19: The histograms of CH1 → CH2 and CH2 → CH1 colocalization value with artifact correction.