# **User's guide of QC-STORM**

## 1. Requirements

**Operation system:** Windows 7 sp1 or newer, 64-bit system.

**Software:**ImageJ or FIJI. Note: please set at least 4GB memory buffer for ImageJ.

**CPU:**CPU memory >= 8GB

**GPU:**Nvidia GPU, Compute capability >= 3.5, GPU memory >= 3GB.

### **Software dependency:**

- 1, download and install Microsoft Visual C++ 2015 Redistributable Update 3 (x64).
- 2, please upgrade your GPU driver to the newest (Compatible Driver Versions >=
- 411.31) or the plugin can't work successfully.
- 3, Download and install Matlab 2020b or mrc.

#### 2. How to install

NanoStitcher is built for ImageJ independently. To install, simply copy the contents of the corresponding folder to the corresponding FIJI folder.

Copy the contents in the "jars" folder in the "jars" folder of FIJI.

Copy the contents in the "plugins" folder in the "plugins" folder of FIJI.

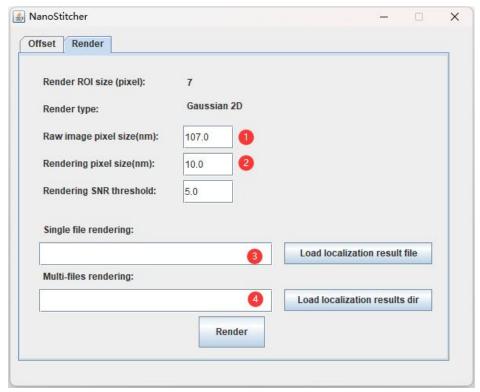
Copy the contents in the "root" folder in the root directory of FIJI.

#### 3. How to use

The NanoStitcher for ImageJ are used for offline processing.

|   |                 | Load dataset dir 1        |
|---|-----------------|---------------------------|
| Number of localization list in t                | he x_direction: | 2                         |
| Number of localization list in the y_direction: |                 | 3                         |
| Extent of overlap between da                    | tasets:         | 4                         |
| Offset storage location:                        | ,               |                           |
|   |                 | Offset storage location 6 |
|   |                 |                           |
| Storage location for stitching                  | results:        |                           |
| Storage location for stitching                  | results:        | Storage stitching resu6   |

- ① Only binary files are stored in this folder, and these binary files are named in the order of stitching from 1 to N.
  - (2) Number of binary files in the x direction.
  - (3) Number of binary files in the y direction.
  - 4 Extent of overlap between datasets.
- (5) The storage location of the first generated file, which stores the offset of each localization lists.
  - 6 Storage location of large localization lists after stitching.
- 7 Start stitching after pressing the button. If the button turns gray, stitching is in progress



- 1 Raw image pixel size(nm)
- 2 Rendering pixel size(nm)
- (3) Fill in or select the single localization binary file to be rendered
- 4 Fill in or select the folder of multiple localization binary files to be rendered Only one of the fourth anf fifth needs to be filled in.

## 4. Localization data

Localization binary files are included in the input and output of each stitch.

There are 12 single-precision floating point number parameters for each molecule:

| Order | Description             |
|-------|-------------------------|
| 1     | Peak intensity (photon) |
| 2     | X (pixel)               |
| 3     | Y (pixel)               |
| 4     | Z (nm)                  |

| 5  | Gaussian PSF Sigma along X-axis (pixel)         |
|----|---|
| 6  | Gaussian PSF Sigma along Y-axis (pixel)         |
| 7  | Total intensity (photon)                        |
| 8  | Background intensity of each pixel (photon)     |
| 9  | SNR calculated by peak intensity and background |
| 10 | Localization precision along X-axis (nm)        |
| 11 | Localization precision along Y-axis (nm)        |
| 12 | Frame number                                    |

# 5. Samples

In the "Samples" folder, the "2x2" folder contains the localization binary files and the "NanoStitcherResult" folder contains the final stitching and rendering result.

Stitching result's parameters

| 8 1   |    |
|---|----|
| Number of localization list in the x-direction. | 2  |
| Number of localization list in the y-direction. | 2  |
| Extent of overlap between datasets.             | 50 |

Rendering result's parameters

| Raw image pixel size(nm):       | 107.0 |
|---------------------------------|-------|
| Rendering image pixel size(nm): | 10.0  |
| Rendering SNR thrshold:         | 5.0   |