User's guide of QC-STORM

July 13, 2019

1. Key features

Feature	QC-STORM for Micro-Manager	QC-STORM for ImageJ
MLE Localization: 2D, Astigmatism 3D	✓	✓
Multi-emitter fitting for both 2D and 3D	✓	✓
Consecutive fitting (temporal grouping) for molecules emitting in adjacent frames	✓	✓
Real-time rendering and statistical information analyzing	✓	✓
Lateral drift correction by cross-correlation		✓
Online feedback control	✓	
Multi-FOV acquisition	✓	

2. Requirements

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

Software: ImageJ or FIJI, Micro-Manager 2.0 beta3. Note: please set at least 4 GB memory buffer for ImageJ, Micro-Manager and ImageJ of Micro-Manager.

CPU: CPU memory >= 8 GB

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

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. How to install

QC-STORM is built for ImageJ and Micro-Manager independently. To install, simply copy corresponding dynamic link library (DLL) files into the ImageJ and Micro-Manager installation folder. And then copy corresponding .jar plug-in file to *plugins* folder of ImageJ and *mmplugins* folder of Micro-Manager respectively.

4. How to use

The QC-STORM for ImageJ and Micro-Manager are used for offline and online processing respectively. They both get 16 bits gray-scale images as input, and output a float super-resolution TIFF image and binary localization data file. The QC-STORM plug-in GUI for ImageJ and Micro-Manager are shown in figure 1-2. The parameters in all panels are shared and only necessary parameters are used.

QC-STORM for ImageJ

After installation, open images by ImageJ, and open QC-STORM in the *Plugins* menu. After proper parameters are set, click Start localization button to process the images. For post-processing, jump to the post-processing panel, and load a binary localization data file and click processing button in the panel.

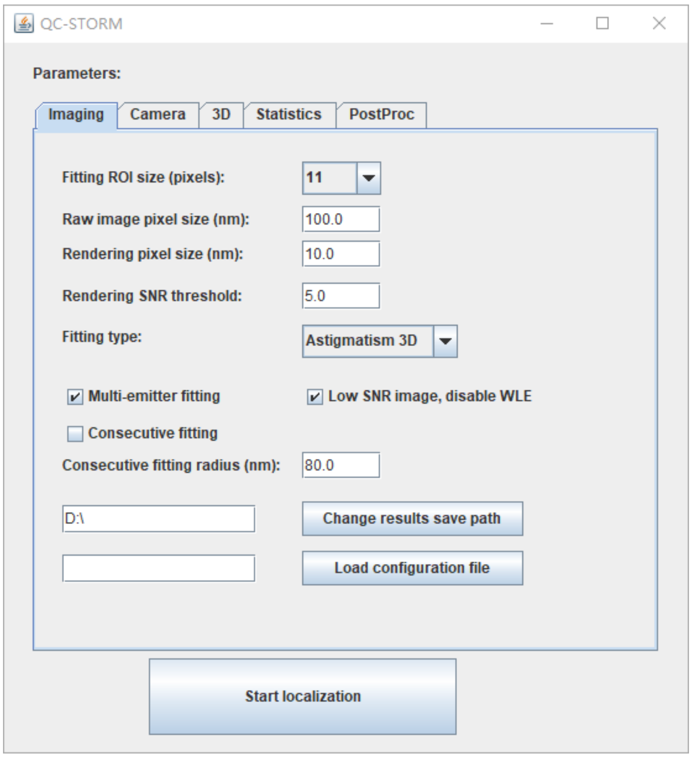

Figure 1. QC-STORM GUI for ImageJ.

QC-STORM for Micro-Manager

First, you may need to create a configuration file for your camera in Micro-Manager to support its acquisition. After installation, the QC-STORM plug-in will be shown in the

Micro-Manager menu plugins/On-the-fly Image processing. You can add, remove or enable and disable QC-STORM in the *On-the-fly processor pipeline* window (Figure 3). If the QC-STORM is added and enabled in the pipeline, the captured images in *Live*, *Snap* and *Multi-Dimensional Acquisition* mode will be processed. We provide a *New live acquisition* to clearly existing captured results.

For default *Live* mode of Micro-Manager, only the displayed images are processed by online processing pipeline, which are only a small part of wholly captured images. To realize full frame rate processing without saving raw images, we provide a *Burst acquisition live* button. If you want to save raw images, we provide a save raw image option, or *Multi-dimensional acquisition* can be used. For Multi-dimensional acquisition, you should set a large enough memory buffer for Micro-Manager to avoid memory overflow.

The feedback control and multi-FOV acquisition are hardware specific, thus you should modify corresponding codes to support your own hardware. It should be easy since QC-STORM control the devices by serial ports.

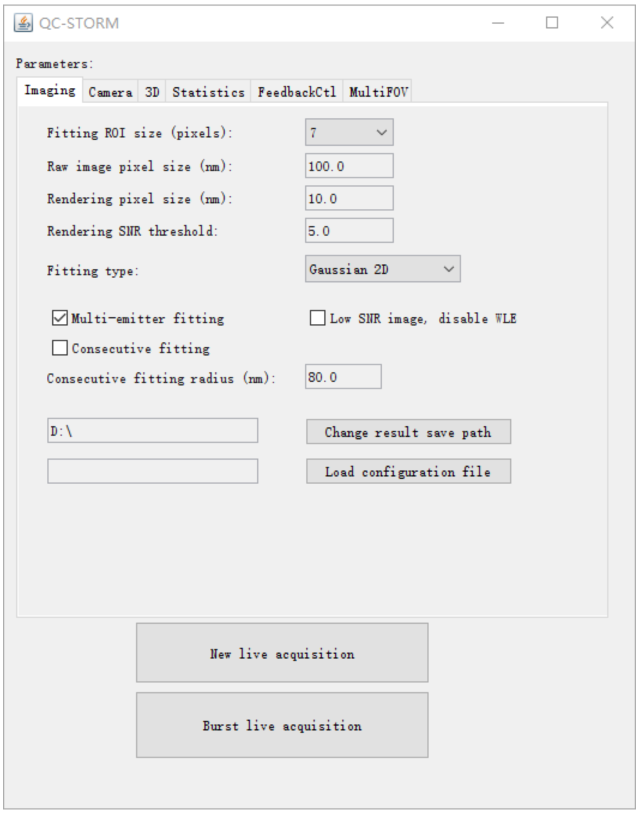

Figure 2. QC-STORM GUI for Micro-Manager.

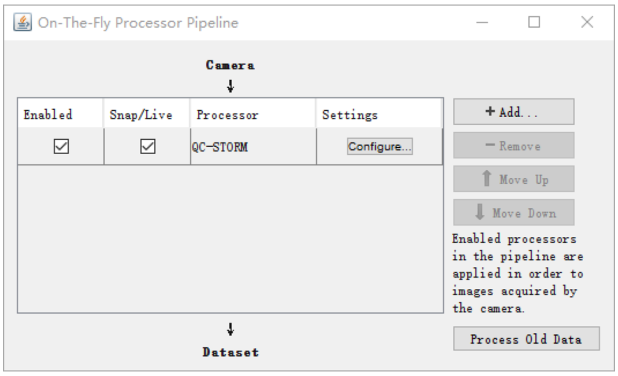

Figure 3. Manage QC-STORM in the On-The-Fly Processor Pipeline of Micro-Manager.

5. Parameter descriptions of QC-STORM

Item	Description
Fitting ROI size (pixels)	Width of extracted molecule ROIs.
Raw image pixel size (nm)	The raw image pixel size in the object plane of imaging system.
Rendering pixel Size (nm)	The rendered super-resolution image pixel size.
Rendering SNR threshold	Molecules with peak signal to noise ratio (SNR) larger than this threshold will be rendered in the super-resolution image.
Fitting type:	PSF fitting type, such as Gaussian 2D, astigmatism Gaussian 3D.
Multi-emitter fitting	Using multi-emitter model to fit the extracted ROI when single-molecule model is not enough. **Note: This function is only suggested for high SNR images. And the single-molecule fitting can already tolerate middle level high density activation.**
Consecutive fitting	Merging molecules continuously in adjacent frames by localization precision weighted average. This selection must be enabled to measure on time.
Low SNR image, disable WLE	We use WLE to improve precision of MLE. **For low SNR (SNR<10) images, this may not works well. For high SNR images, we suggest using WLE.**
Change result save path	Set a path to save your results. The C disk is not recommended.
Load configuration file	Load an existing configuration file to update parameters in the panel.
Change result save path	Configure where super-resolution image and binary localization data will be saved.
QE	Camera Quantum efficiency at the working wavelength.
Kadc (e-/DN)	Electron number per pixel gray value. For EMCCD, please remove the EM gain and consider the EMCCD as

	a normal camera.
Offset (DN)	Average camera pixel value when there is no light.
Z depth rendering range	Maximum Z depth of 3D imaging to control color coded image rendering. Depth outside this range will also be rendered.
Z depth correction factor	The final Z depth calculated by calibration curve will multiplied by this factor to correct refractive index mismatch.
px4,px3, px2, px1, px0	The four order polynomial calibration curve used in astigmatism 3D imaging, which is measured by: $d = SigmaX^2 - SigmaY^2$ $Z = p4 \times d^4 + p3 \times d^3 + p2 \times d^2 + p1 \times d^1 + p0$ We provide Matlab codes to measure the calibration curve parameters. To use the code, first select images of beads within Z depth where the PSFs don't spread into two blobs, and then process the image by our plug-in without setting the correct calibration curve parameters. Finally calculate the localizations by the Matlab codes to generate the calibration curve parameters. We fit the calibration curve independently for SigmaX > SigmaY and SigmaX < SigmaY.

6. Localization data

After each processing, the results including a super-resolution image and a localization binary data file will be saved in the result path. We provide MATLAB codes to read the binary data file, and an executable file to convert the binary data into .csv file. 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