**General instructions on how to use WindSTORM (MATLAB version)**

This is the source code of WindSTORM, a high-speed high-density emitter localization software. The software is written in MATLAB. There are four testing datasets included in the “data” folder.

**Hardware requirements**

RAM: For the input dataset, we recommend a memory size of 16-32 GB.

**Software requirements**

MATLAB R2015b and higher (tested on MATLAB R2015b and R2018a Windows 7 and 10); Image processing toolbox.

The master code is “WindSTORM.m” to help users get started.

**Input files**

An input dialog box will be popped up to ask users to enter the following 6 parameters:

**PathName:** the file path of the image data. The default is ‘.\data’.

**FileName:** the file name of the image sequence, with any format that can be recognized by “imread” in MATLAB. The default is ‘EPFL\_Microtubule\_Alexa647.tif’, the reference dataset downloaded from <http://bigwww.epfl.ch/smlm/datasets/index.html?p=real-hd>).

**Intensity:** the estimated average emitter intensity of the dataset. The default is 10000 based on the reference dataset.

(**Tip:** To estimate the average emitter intensity of the dataset, we recommend that the users can run just a single image frame with the single-emitter fitting method (e.g., ThunderSTORM) and use the average intensity from the reconstructed image as the estimated value.)

**Sigma:** the kernel width of the point spread function (PSF) of the system. We recommend the value of 1.2 to 2.0 pixels to balance the sampling rate and signal-to-noise ratio (SNR).

(To estimate the kernel width of PSF, the users can take a single image frame, select a single isolated emitter and measure the width of the PSF.)

**Count2photon:** the conversion ratio from photon counts recorded on the camera to number of photons.

**Baseline:** the photon baseline level of the camera.

(Both Count2photon and Baseline are camera-specific parameters.)

**Note 1:** For the current version, the image size must be adjusted to be one of the following: 64x64; 128x128; 256x256; 512x512; 1024x1024. Future updates will be provided to process the image of any size.

**Note 2:** If a large image size or long image sequence is used, the 32 GB RAM may be required (e.g., testing dataset “Cell\_Microtubule\_Alexa647.tif” and “Tissue\_Chromatin\_Alexa647.tif”).

**Note 3:** The examples to set parameters for other datasets (Cell\_Microtubule\_Alexa647.tif, Cell\_Microtubule\_mEos32.tif and Tissue\_Chromatin\_Alexa647.tif”) were provided.

**Note 4:** For users who use older version of MATLAB that does not support “inputdlg” command, the examples are provided for each dataset. The users need to uncomment the code that sets the above 6 parameters.

**Computational time**

We display the computational time for WindSTORM (not including the data read/write time) on the MATLAB.

**Output file**

The output file is a cvs file that shows 6 columns: (emitter) id, frame, (emitter) x position (in pixel), (emitter) y position (in pixel), intensity (photons). The users can import this file into ThunderSTORM for visualization. The users need to specify the pixel size.

(For the testing dataset, the pixel size for “EPFL\_Microtubule\_Alexa647.tif” is 100 nm; the pixel size for “Cell\_Microtubule\_Alexa647.tif”, “Cell\_Microtubule\_mEos32.tif” and “Tissue\_Chromatin\_Alexa647.tif” is 81 nm.)

**Note 5:** **No drift correction** is performed on the data output. For dataset with a long image sequence (e.g., the testing dataset “Cell\_Microtubule\_Alexa647.tif” and “Tissue\_Chromatin\_Alexa647.tif”), the users need to use cross correlation-based drift correction method (e.g., this method was built in ThunderSTORM) to correct for system drift.

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