

Tutorial for analyzing single-channel SMLM data using SMAP

Tested on Matlab 2021b.

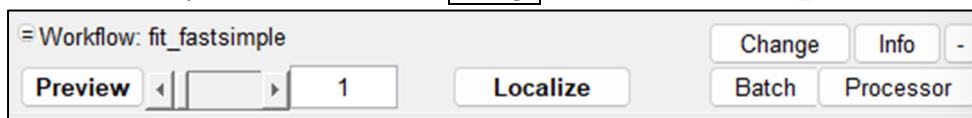
Future issues will be fixed in the develop branch at <https://github.com/jries/SMAP>.

Generate the PSF model (.h5) file using uiPSF.

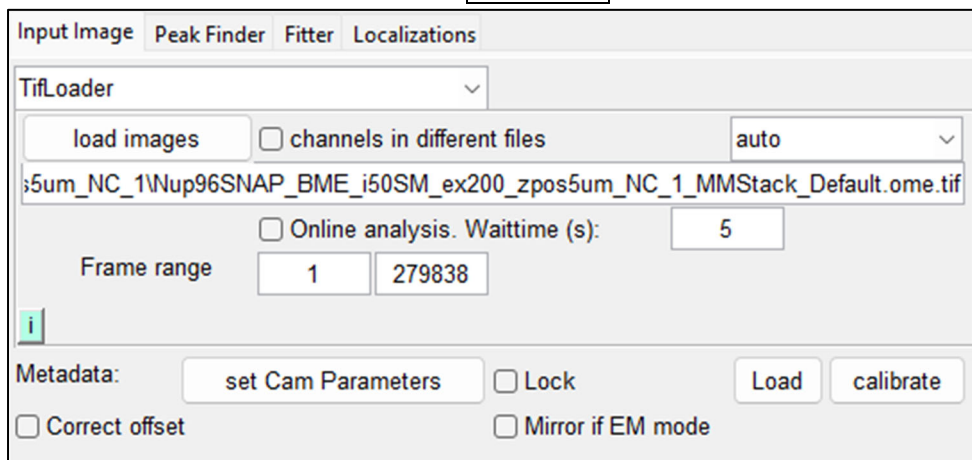
- If the data is in .tif format, set 'swapxy = true'. This is because there is a permutation in SMAP when loading .tif files.

Then follow the steps below for localization in SMAP.

1. Open MATLAB, set the working directory to C:\Users\Ries Lab\git\SMAP\
2. In Matlab command window, run: SMAP
3. In SMAP GUI, open tab Localize, click **Change** and select the file 'fit_fastsimple.txt'.



4. Go to tab Localize -> Input Image, click **load images** and select the first .tif file in the data folder.



5. Edit parameters defined in **set Cam Parameters** if metadata was not available. Sometimes, the bead data and the SMLM data are mirrored when they were collected at different EM mode settings, in this case, check 'Mirror if EM mode'. If insitu PSF model is used, uncheck 'Mirror if EM mode'.
6. Open tab Localize -> Fitter, click **Load 3D cal**, select the .h5 file from uiPSF. Set 'Iterations' to 50. Check 'RI mismatch' if the PSF model was generated from bead data.

Input Image Peak Finder Fitter Localizations

ROI size (pix) 13 0

Spline Iterations: 50 ROIs/fit: 15000

Load 3D cal E:\EMBL files\data 2022\230202_ z0(nm) 0

☐ additional Gauss fit

☐ New pixelsize X,Y (um): ☐ RI mismatch: .8

☐ sCMOS

☐ get asymmetry

7. In Localize -> Peak Finder, click **Preview** and adjust 'dynamic (factor)' based on the output image.

Input Image Peak Finder Fitter Localizations

DoG s: 1.2

☐ correct sCMOS variance

dynamic (factor) 1.7

☐ minimum distance (pix) 7

1. Then click **Localize**.
9. After localization is finished, in panel 'format', click **Reset** at the upper right of the SMAP GUI. Go to tab Process -> Drift, and set 'pixrec nm' to 30 and 'z binwidth nm' to 20. And set 'timepoints' and 'timepoints' z according to the length of the data. Set 'zrange nm' according to the axial range of the data.

Drift Register Assign2C Modify

drift correction X,Y,Z
showdriftinfo

Info

☒ Show results

Run

☒ Correct xy-drift

timepoints 30 pixrec nm 30

window pix 7

maxdrift nm 1000

max size (pix) 4096

☒ Correct z-drift

timepoints z 30 z binwidth nm 20

z fit window pix 9

zrange nm -800 800

slice width nm 200

10. In the menu, go to Plugins -> Process -> Drift -> driftcorrection_dme. Check 'use median CRLB'. Set 'zrange nm' according to the axial range of the data. Then click **Run**.

Info

Run

☒ Show results

☒ Correct z-drift

frames/bin

100

max neighbours

10000

zrange nm

-800 800

☒ use median CRLB

☒ use GPU

11. Go to tab 'Render' for rendering super-resolution image.