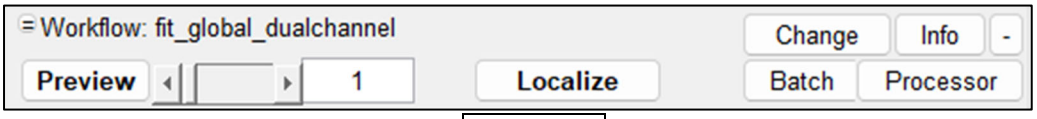
Tutorial for analyzing data from dual-color ratiometric imaging using SMAP

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.

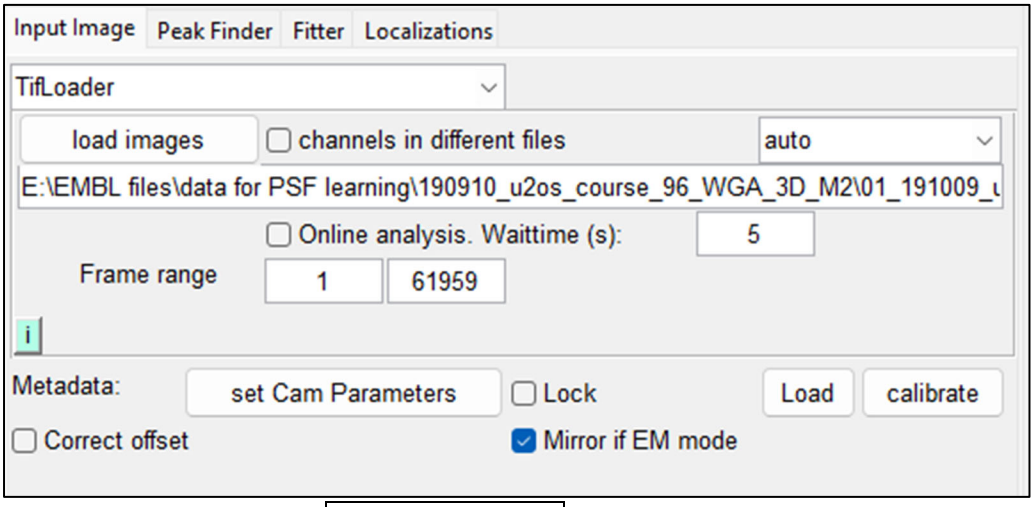
- If the data is in .tif format, set 'swapxy = true'. This is because there is a permutation in SMAP when loading .tif files.
- Set 'ref_channel = 0', this does depend on which channel SMAP consider as the reference channel, usually it should be set to zero.

Then follow the steps below for localization in SMAP.

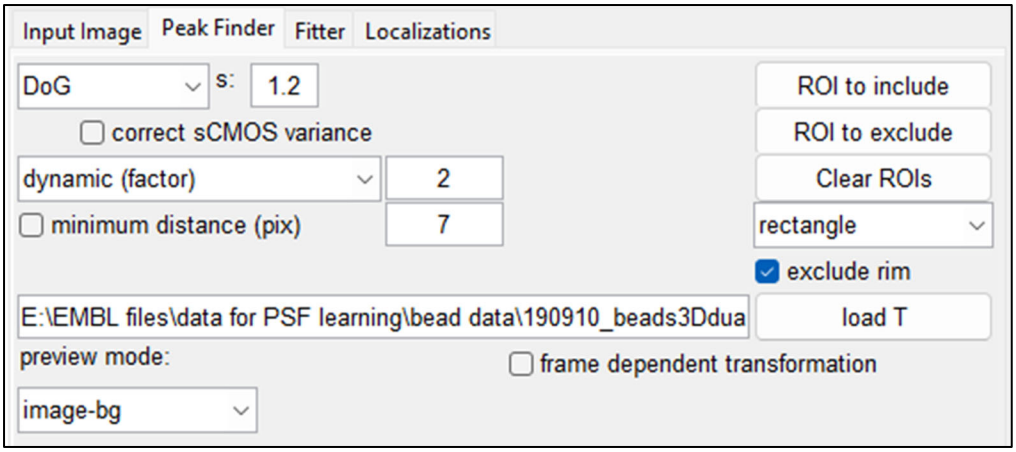
1. Open MATLAB, set the working directory to C:\Users\Ries Lab\git\SMAP\
2. In Matlab command window, run: SMAP
3. In SMAP GUI, open tab Localize, click Change and select the file 'fit_global_dualchannel.txt'.



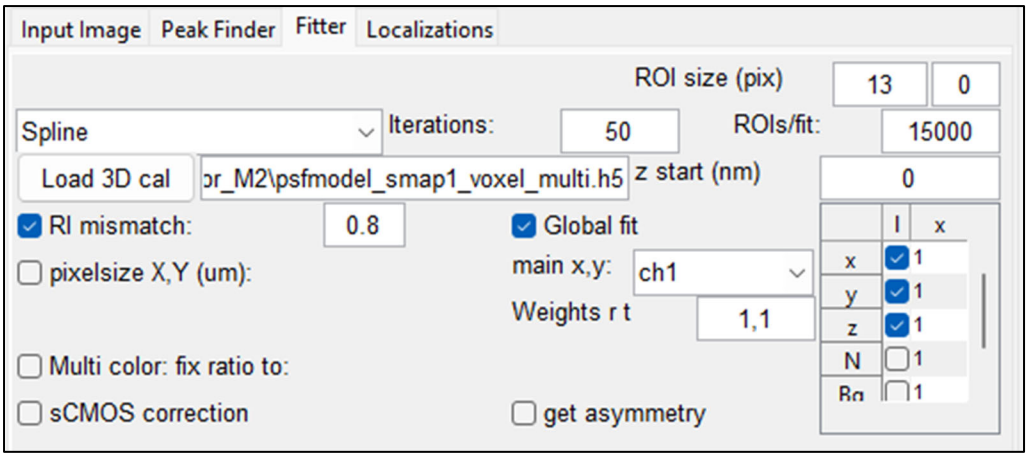
4. Go to tab Localize -> Input Image, click load images and select the first .tif file in the data folder.



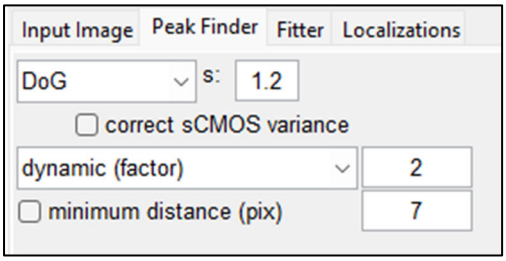
5. Edit parameters defined in set Cam Parameters if metadata was not available. Sometimes, the bead data and the SMLM data are mirrored when they were collected at different EM mode settings, in this case, check 'Mirror if EM mode'. If insitu PSF model is used, uncheck 'Mirror if EM mode'.
6. Open tab Localize -> Peak Finder, click load T, select the .h5 file from uiPSF.



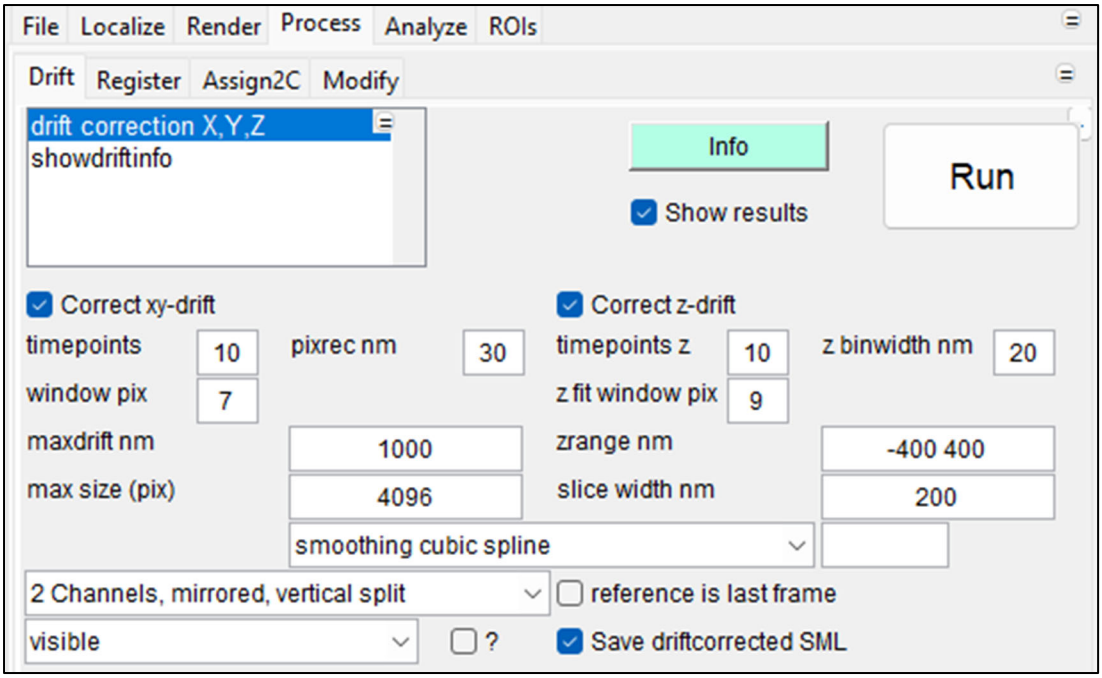
7. Open tab Localize -> Fitter, click Load 3D cal, select the same .h5 file. Check 'Global fit' and select 'ch1' for 'main x,y'. Check 'RI mismatch' if the PSF model was generated from bead data.



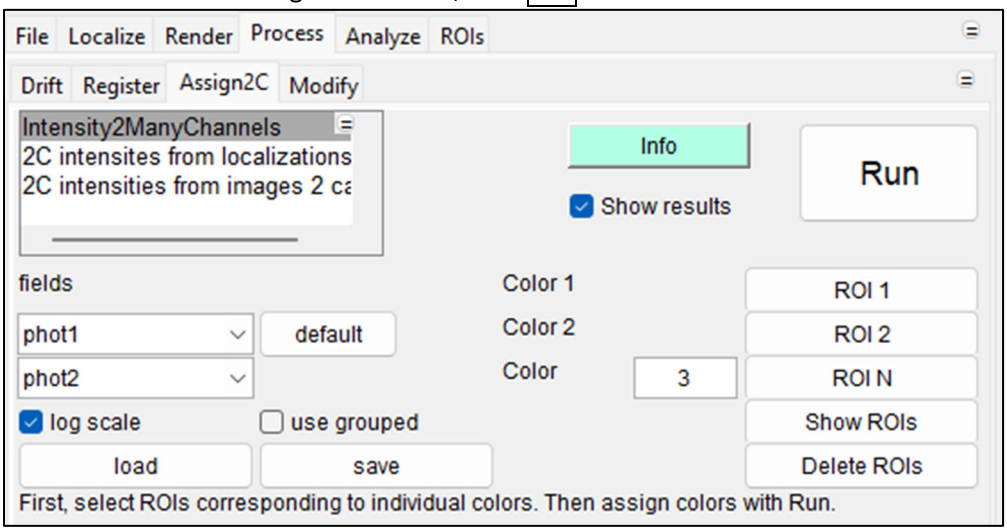
8. In Localize -> Peak Finder, click Preview and adjust 'dynamic (factor)' based on the output image.

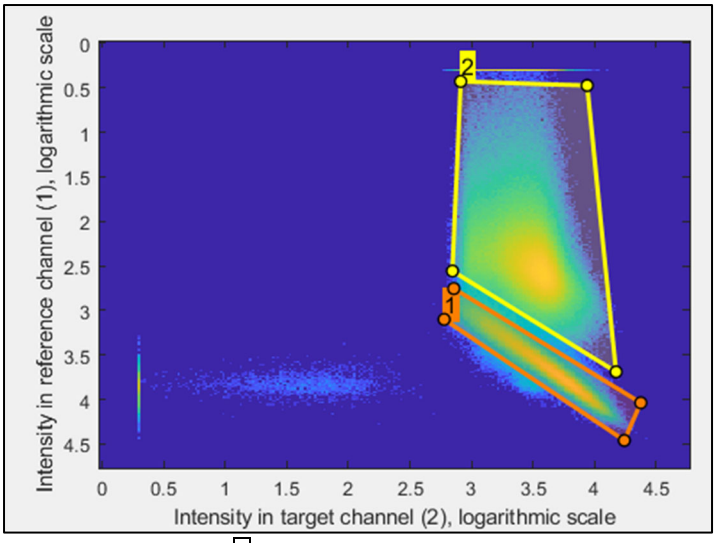


9. Then click Localize.
10. After localization is finished, in panel 'format', click Reset at the upper right of the SMAP GUI.
11. Go to tab Process -> Drift, and set 'pixerec nm' to 30 and 'z binwidth nm' to 20. In the bottom dropdown menu, select correct splitting for your data, e.g. '2 Channels, mirrored, vertical split' Then click Run.

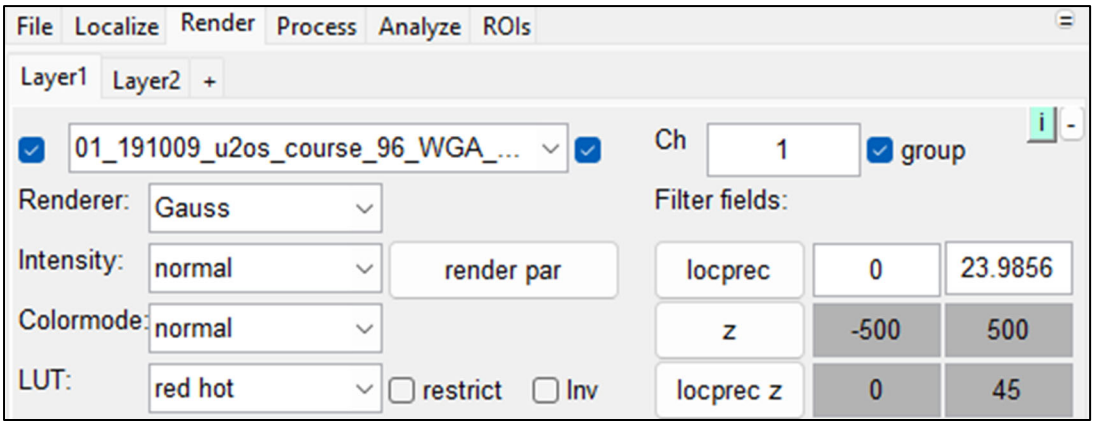


12. Go to tab Process -> Assign2C, select 'Intensity2ManyChannels'. Click Show ROIs, then click ROI 1, draw the region for color channel 1 in the popup Figure, then click ROI 2, draw the region for color channel 2. After region selection, click Run.





13. In tab Render, click + to create one more layer and set 'Ch' to 1 for Layer1 and to 2 for Layer2. For different layer, set 'LUT' to different colormap.



14. Now you can render a dual-color super-resolution image.