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1. Introduction

The MATLAB app allows to evaluate single molecule localization microscopy data in the form of tiff image stacks (time series) taken in a total internal reflection fluorescence (TIRF) microscope. It estimates 3D position, signal and background level for each individual molecule.

To avoid localization biases, the app allows the user to measure optical aberrations present in the microscope by taking a z-stack of a small (e.g. 100 nm) fluorescent bead. This feature makes it also possible to evaluate data taken with engineered PSFs (e.g. using a cylindrical lens).

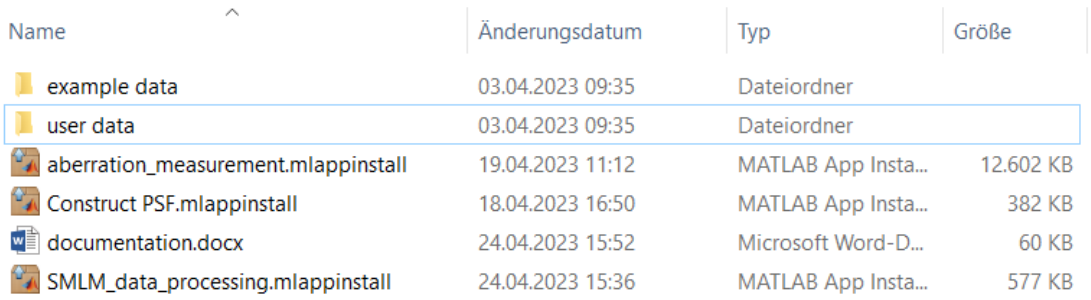
The app requires the ImageJ plugin "Thunderstorm" for doing a coarse, 2D pre-localization.

The app consists of three parts that can be also run individually:

„SMLM_data_processing.mlapp“	The main app
“construct_PSF.mlapp”	A sub-app to construct point spread functions, which can be loaded into and used in the main app
“aberration_measurement.mlapp”	A sub-app to estimate pupil phase aberrations from a previously recorded z-stack of a single fluorescent bead

2. Installation

Unpack the zip archive and double click on the apps to install them in Matlab. They will appear in the MATLAB “apps” bar. **When executing the apps, be sure that the Matlab path is set to the path where the folders “example data” and “user data” are located**. The latter contains files that characterize the PSF, objective lenses, cameras and aberrations. Otherwise, the app fails to load the default PSF at startup.



3. SLM_DATA_PROCESSING

3.1. General workflow

The app consists of 3 tabs: "**define PSF**", "**prelocalization**" and "**precise localization**". The general workflow comprises:

- the definition of the PSF that persisted when the raw image data was recorded (in tab: "define PSF")
- evaluating the raw image data in 2D using Thunderstorm and saving the result as csv-table. Note that the correct pixel size must be set in Thunderstorm under "camera settings". The other parameters there are not relevant.
- loading the raw image data + Thunderstorm csv-table into the app (in tab: "prelocalization")
- performing precise 3D localization (in tab: "precise localization")

3.2. Detailed explanations to the app

Fig. 3.1 **Error! Reference source not found.**shows the "define PSF" tab as it should appear at startup.

The text box contains info about the PSF such as name, defocus value, oversampling value, the used camera and objective lens (explanations to these parameters can be found in chapter xxx). The figure on the right allows one to inspect the PSF for all z-values it supports. The z-value is defined as the position of a molecule form the coverslip and ranges from 0 to $\lambda_{em}/3$ (a third of the peak emission wavelength).

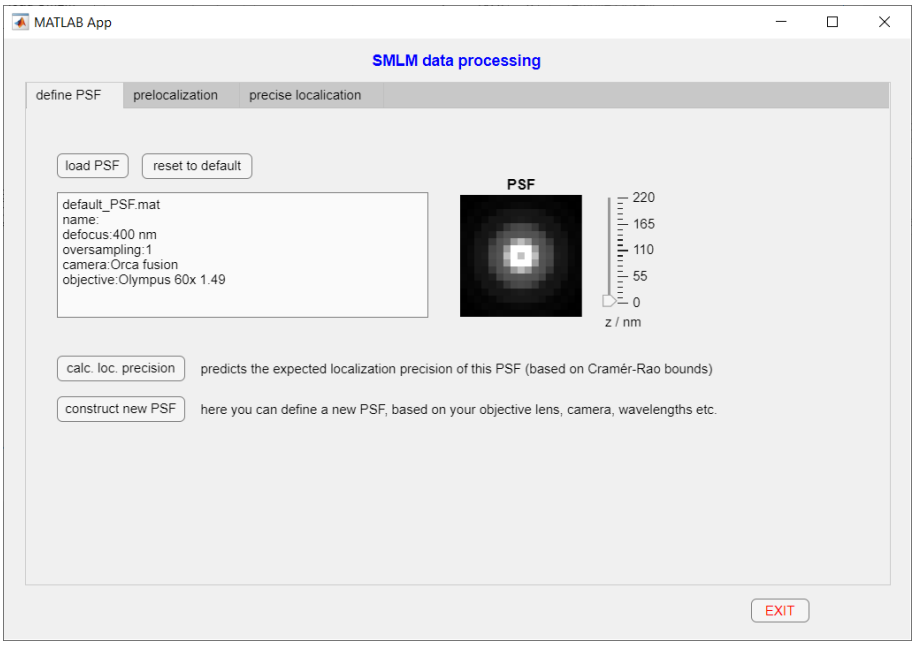


Fig. 3.1 The "define PSF" tab of the main app. It allows the user to load and inspect point spread functions (PSF) created by the app "construct_PSF.mlapp".

reset to default	loads the default PSF. To replace the default PSF by a user-defined one, you have to construct it in “construct_PSF.mlapp” and save it as “default_PSF.mat” in the folder “.\user data\PSFs”.
calc. loc. precision	displays the expected localization precision of the PSF using Cramér Rao lower bounds.
construct new PSF	launches the app “construct_PSF.mlapp”

Error! Reference source not found. shows the “prelocalization” tab as it should appear at startup.

Fig. 3.2 The „prelocalization“ tab of the main app. Here you can load the raw image stack and 2D localization results from Thunderstorm.

load SMLM image stack	For importing the raw image stack in tiff format
load TS data (csv) & crop molecules	For importing the results table created in Thunderstorm. The table should contain the columns “id”, “frame”, “x / nm”, “y/nm”. After data import, single molecule images are cropped from the raw image stack, using the position information in the Thunderstorm table. Therefore, the correct pixel size must be also set in Thunderstorm under “camera settings”.
show random molecule	Shows a random molecule to verify if they have been cropped correctly: The molecule should appear in the center of the small image.
remove closely spaced molecules	Removes molecules detected in Thunderstorm that are too closely spaced. A threshold (minimum) distance can be specified in “d_min (AU)”, which is given in Airy units (AU = 1.22 * λ_{em}/NA)
save preloc data	Saves the stack of cropped molecules and the table as mat-file. The mat-file contains two variables, a 3D array (“images”) and a table (“positions”), e.g. like this: Name ▲ Value images 17x17x21090 double positions 21090x4 table
load preloc data	Previously generated preloc data can be loaded here. The data can then be processed in the “precise localization” tab. It can also be filtered again using “remove closely spaced molecules”.

Fig. 3.3 shows the “precise localization” tab as it appears at startup.

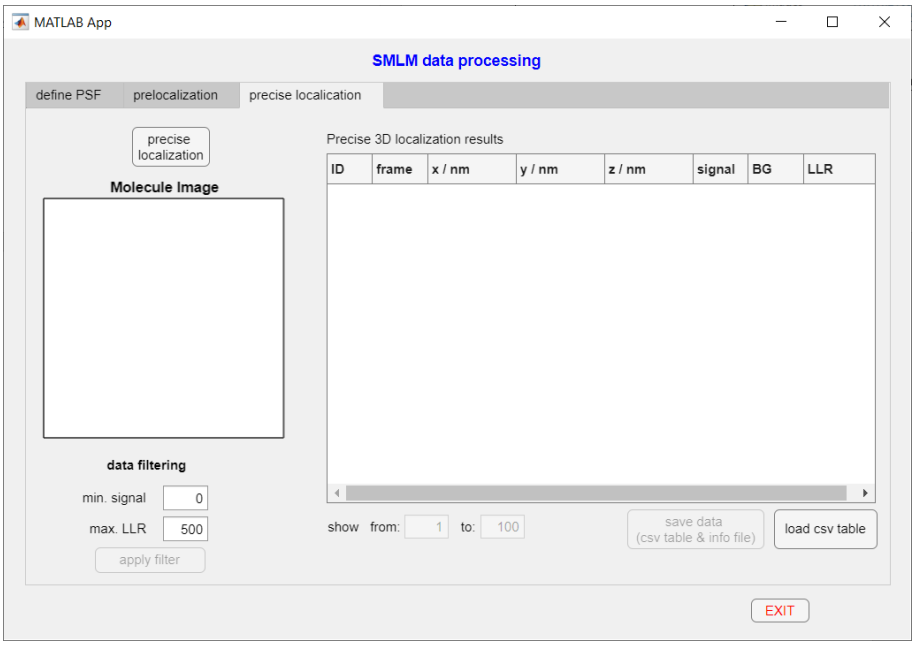


Fig. 3.3 The "precise localization" tab

Precise localization	Performs 3D fine localization using a Levenberg Marquardt algorithm and maximum likelihood estsimation for Poissonian noise.
Precise 3D localization results	The table is automatically filled afer the precise localization has finished. Description of columns: “**ID**” and “**frame**” denote the molecule identification number and the raw image stack frame number which contains the molecule. The data is copied from the Thunderstorm table. **x/y/z** contain estimated molecule positions in nm. **signal/BG** contain estimated signal and background level in photons. Note that the photon number corresponds to the estimated number of photons that are collected and transmitted by the objective lens (NOT the number of photons in the actual single molecule image, which is smaller) **LLR** Log-Likelihood ratio; this provides a measure for the fit quality. The smaller this number the better the fit.
Data filtering	Allows basic data filtering. A minimum value for the signal (in photons) and a maximum number for the LLR value can be defined. Note that “apply filter” must be clicked in order to perform the filtering process.
save data (csv table & info file)	Saves the shown table “precise 3D localization results” in csv format and a mat file containing the used PSF. Note that also info about the used camera and objective lens are part of the PSF definition, so this info is stores as well.
load csv table	For importing and visualizing previously processed data, or data that has been drift-corrected in Thunderstorm.

3.3. Drift correction

The app cannot correct the data for x-y drifts that occurred during the measurement. For drift correction, follow these steps:

- Export the final data table by clicking on “save data (csv table & info file)”
- Import the csv table in Thunderstorm and perform the drift correction there

- Export from Thunderstorm again and import data in the app for visualization