Instructions Manual for Integrated Localization Environment (ILE)

Content:

Overview

Integrated Localization Environment (ILE) is a plug-in for Matlab to evaluate and process single molecule localization microscopy (SMLM) data. It is a running project, which means that further routines can be added in future. If you have suggestions or find any bugs, please let us know.
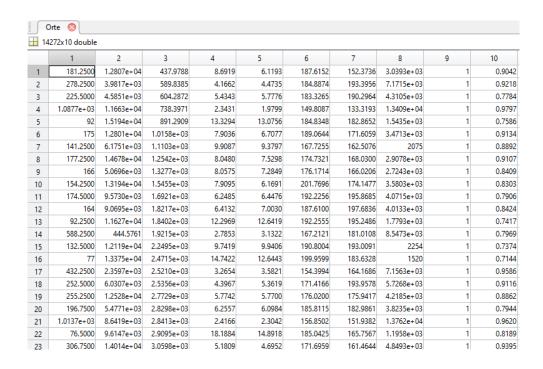
Requirements:
- Matlab 2012a or higher
- Image Processing Toolbox
- Statistics and Machine Learning Toolbox
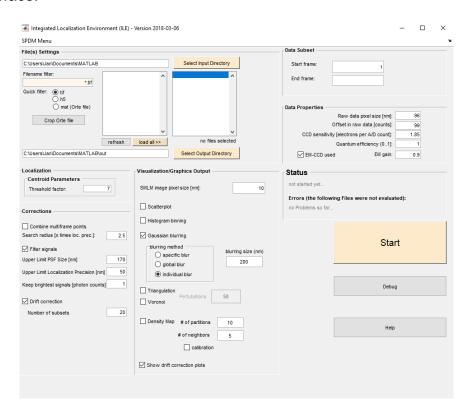
Supported Input File Formats:
- Tagged Image File Format (.tiff)
- Hierarchical Data Format (.h5, as output by PYthon Microscopy Environment (PYME))
- MAT-file (list of localizations in *Orte* format)

Format of *Orte* file

- Column 1: Maximum of signal peak in photons
- Column 2: x-coordinate of signal
- Column 3: y-coordinate of signal
- Column 4: localization precision in x-direction
- Column 5: localization precision in y-direction
- Column 6: width of PSF in x-direction
- Column 7: width of PSF in y-direction
- Column 8: accumulated intensity in photons
- Column 9: frame number
- Column 10: accumulated intensity of signal after signal separation divided by intensity of signal before signal separation

	1	2	3	4	5	6	7	8	9	10
1	181.2500	1.2807e+04	437.9788	8.6919	6.1193	187.6152	152.3736	3.0393e+03	1	0.9042
2	278.2500	3.9817e+03	589.8385	4.1662	4.4735	184.8874	193.3956	7.1715e+03	1	0.9218
3	225.5000	4.5851e+03	604.2872	5.4343	5.7776	183.3265	190.2964	4.3105e+03	1	0.7784
4	1.0877e+03	1.1663e+04	738.3971	2.3431	1.9799	149.8087	133.3193	1.3409e+04	1	0.9797
5	92	1.5194e+04	891.2909	13.3294	13.0756	184.8348	182.8652	1.5435e+03	1	0.7586
6	175	1.2801e+04	1.0158e+03	7.9036	6.7077	189.0644	171.6059	3.4713e+03	1	0.9134
7	141.2500	6.1751e+03	1.1103e+03	9.9087	9.3797	167.7255	162.5076	2075	1	0.8892
8	177.2500	1.4678e+04	1.2542e+03	8.0480	7.5298	174.7321	168.0300	2.9078e+03	1	0.9107
9	166	5.0696e+03	1.3277e+03	8.0575	7.2849	176.1714	166.0206	2.7243e+03	1	0.8409
10	154.2500	1.3194e+04	1.5455e+03	7.9095	6.1691	201.7696	174.1477	3.5803e+03	1	0.8303
11	174.5000	9.5730e+03	1.6921e+03	6.2485	6.4476	192.2256	195.8685	4.0715e+03	1	0.7906
12	164	9.0695e+03	1.8217e+03	6.4132	7.0030	187.6100	197.6836	4.0133e+03	1	0.8424
13	92.2500	1.1627e+04	1.8402e+03	12.2969	12.6419	192.2555	195.2486	1.7793e+03	1	0.7417
14	588.2500	444.5761	1.9215e+03	2.7853	3.1322	167.2121	181.0108	8.5473e+03	1	0.7969
15	132.5000	1.2119e+04	2.2495e+03	9.7419	9.9406	190.8004	193.0091	2254	1	0.7374
16	77	1.3375e+04	2.4715e+03	14.7422	12.6443	199.9599	183.6328	1520	1	0.7144
17	432.2500	2.3597e+03	2.5210e+03	3.2654	3.5821	154.3994	164.1686	7.1563e+03	1	0.9586
18	252.5000	6.0307e+03	2.5356e+03	4.3967	5.3619	171.4166	193.9578	5.7268e+03	1	0.9116
19	255.2500	1.2528e+04	2.7729e+03	5.7742	5.7700	176.0200	175.9417	4.2185e+03	1	0.8862
20	196.7500	5.4771e+03	2.8298e+03	6.2557	6.0984	185.8115	182.9861	3.8235e+03	1	0.7944
21	1.0137e+03	8.6419e+03	2.8413e+03	2.4166	2.3042	156.8502	151.9382	1.3762e+04	1	0.9620
22	76.5000	9.6147e+03	2.9095e+03	18.1884	14.8918	185.0425	165.7567	1.1958e+03	1	0.8189
23	306.7500	1.4014e+04	3.0598e+03	5.1809	4.6952	171.6959	161.4644	4.8493e+03	1	0.9395

User Interface:

Quick Start Guide

1. Open the input directory of the raw SMLM acquisitions by clicking on the *Select Input Directory* button.

2. Select the appropriate file type of your raw SMLM acquisition. If the correct file type is selected, files will occur in the file list. Furthermore, the list can be filtered by specific file names.
3. To select files for evaluation just click on them and they will be added to the processing file list. If several files are selected they will be batch processed. To remove files from evaluation just click on their file names in the processing file list.
4. Next, select the directory, where you want to save the results of the SMLM evaluation (list of localization, here called *Orte*, reconstructed SMLM images etc.) by clicking on the *Select Output Directory* button.
5. Add the camera properties that were used to capture the data.
6. (Optional) To evaluate only a subset of each image stack, enter the start and end frame. If you want an evaluation until the last frame of each stack, let the field *End frame* empty.
7. In the *Centroid Parameters* panel enter the threshold value that should be used by the localization algorithm. A value of 3 is usually a good starting point.
8. (Optional) To apply correction procedures check or uncheck the corresponding routines (for details see section Components).
9. Finally, enter the pixel size of the reconstructed SMLM image and visualization method that should be used.
10. To start the evaluation, press the *Start* button.

<u>Components</u>
File Settings Panel:

Select Input Directory:	Select directory which contains SMLM raw data localization file directory.
Filename Filter:	Filters data in the selected directory according to filename and file type.
Quick Filter:	Filters data in the selected directory according to the file type. Currently .tif and .h5 file formats are supported. Furthermore, list of localization (following the specifications of *Orte* files with ending *.mat) can be selected and used for correction and visualization routines.
Crop Orte File:	Opens a dialog box, where an *Orte* file can be selected. A SMLM visualization from the Orte file will be opened and the user can draw a rectangle around a region he wants to crop.
Refresh:	Refresh the input file list if a new directory or filter name is entered.
Load all:	Loads all files from the input file list into the processing file list.
Select Output Directory:	Select directory in which the results will be saved. If no directory is entered, the program creates by default a folder called "out" in the directory selected by input file directory.

Data Subset Panel:

Start Frame: Sets the frame number from where the evaluation of the image data stack should start.

End Frame: Last frame for evaluation. If empty, the image data stack will be evaluated until the end.

Data Properties Panel:

Raw data pixel size: Pixel size of the camera in nm.

Offset in raw data: Offset of camera in counts.

CCD sensitivity: Conversion factor to convert from counts to electrons.

Quantum efficiency: Quantum efficiency of camera [0..1]

EM-CCD used   Check if EM-CCD with EM gain is used, uncheck for conventional CCD.

EM gain   EM gain of EM-CCD camera.

Localizations Panel:

Threshold factor: Sets the threshold value for signal extraction by the localization algorithm. A value of 3 is usually a good starting point.

Corrections Panel:

Combine multiframe points: Check if you want to remove points occurring in consecutive frames. You can also specify the radius for multiframe point search in multiples of the localization precision.

Filter signals: Check if you want to filter the list of localizations. Filtering can be done for PSF size, localization precision and maximum intensity of signals.

Drift correction: Check if you want to correct the list of localization for drift. One can specify the number of subsets in which the image data stack should be split.

Visualization/Graphics Output Panel:

SMLM image pixel size: Final pixel size of the reconstructed image in nm.

Scatterplot   Scatterplot representation of point coordinates. Will be saved as Matlab figure.

Histogram Binning   Points are binned into a grid, where grid size is specified by SMLM image pixel size.

Gaussian blurring   Similar to histogram binning, but every point is blurred by a Gaussian. Following blurring methods can be selected:

    Specific blur: Every point is blurred with a sigma specified by the user (blurring size in nm).

    Global blur: Every point is blurred with a sigma equally to the mean x- and y-localization precision of the data set.

    Individual blur: Every point is blurred with a sigma corresponding to its mean x- and y-

	localization precision. (Different localization precisions will be binned. Default number of bins between max. and min. localization precision 100.)
Triangulation	Visualization based on triangulation. Number of perturbations describes the number of iterations, where a new set of localizations is created. In the new set of localizations points are randomly distributed within their corresponding localization precision to smooth the triangles. Resulting image is the sum over all images.
Voronoi	Similar to Triangulation, but with Voronoi cells.
Density map	Every pixel in the reconstructed image represents the local density (mean distance to next neighbours, where number of neighbours is specified). To save memory and accelerate processing, image can be partitioned (Number of points in one partition should be larger than number of neighbours for calculation). If calibration is checked local density is calibrated to number of events per square nm.

| Show drift correction plots: | If checked the calculated drift will be plotted. |

All reconstructed images will be saved as tif. – file, containing the pixel size of reconstruction as metadata. Scatterplots are saved as Matlab figures.

Status Panel:
Shows the status of evaluation and possible errors during runtime.

Latest settings in GUI are saved when closing figure.

Troubleshooting

Error occurs during runtime and start button is disabled:

Click on *SPDM Menu* in the upper left corner of the GUI and press *Enable Start Button*.