WinTILD Quick Manual

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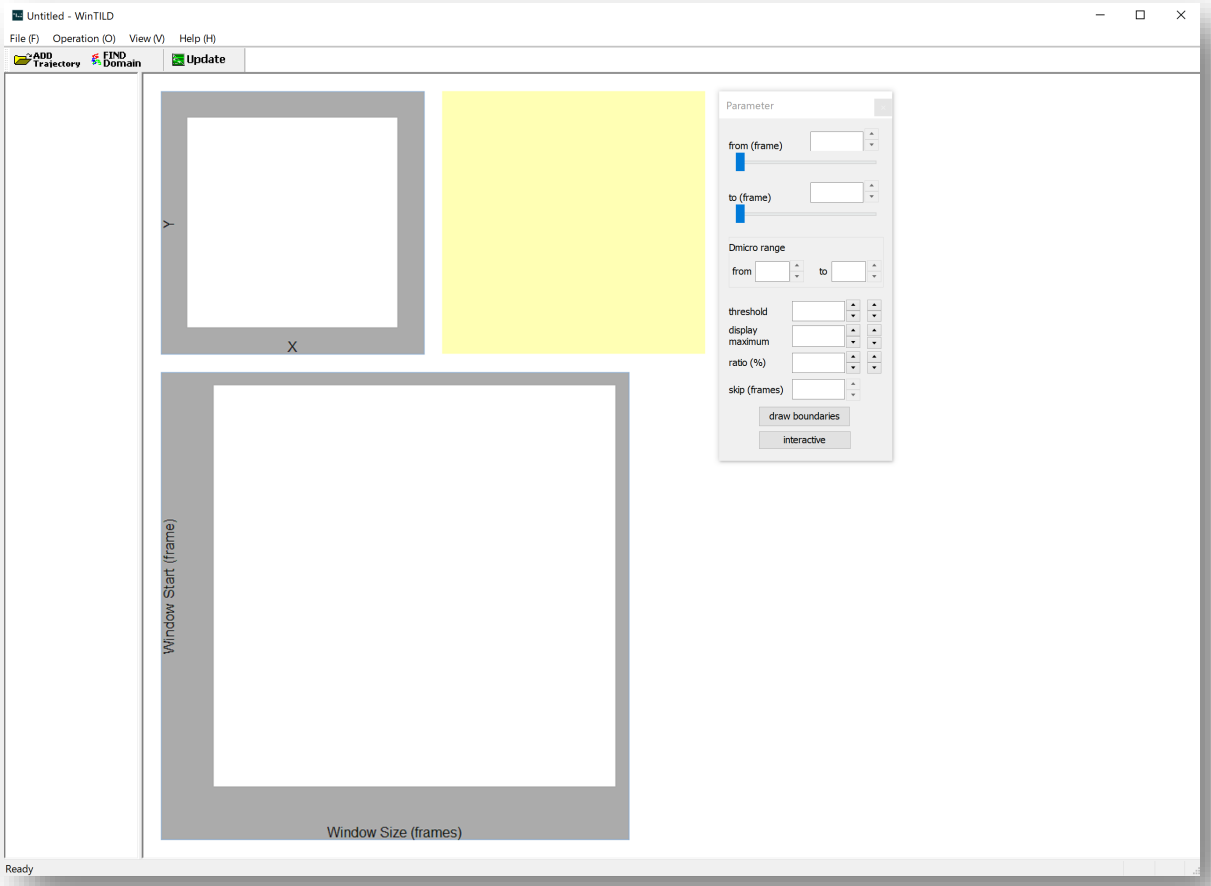
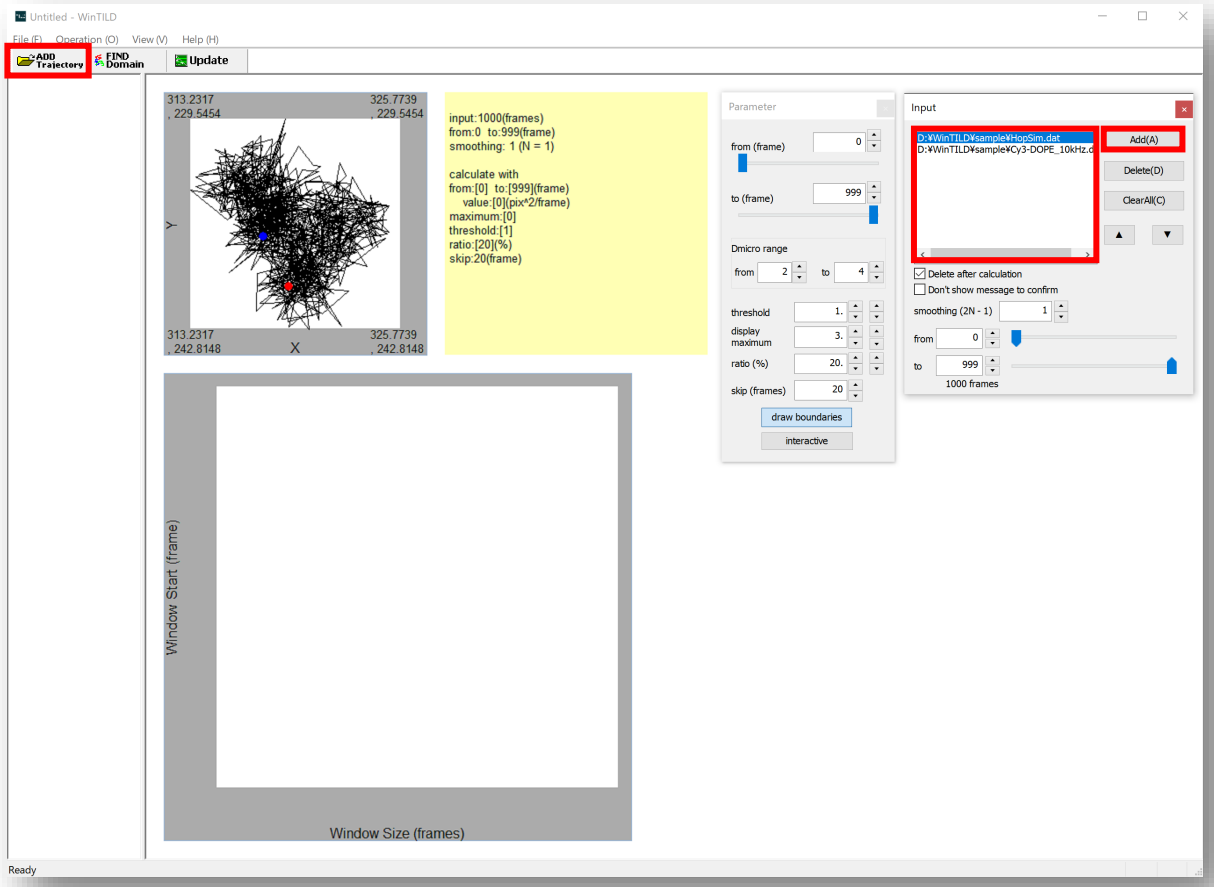
Download and launch the software

- Download WinTLD_64.exe (Windows 64-bit standalone software) from https://github.com/kusumi-unit/WinTILD/releases and locate it in any folder.
- Launch WinTLD.exe. Once launched and operated, an initialization file (WinTLD_64.ini) is generated in the same folder. At the next launch, the setting of the last operation is automatically loaded.



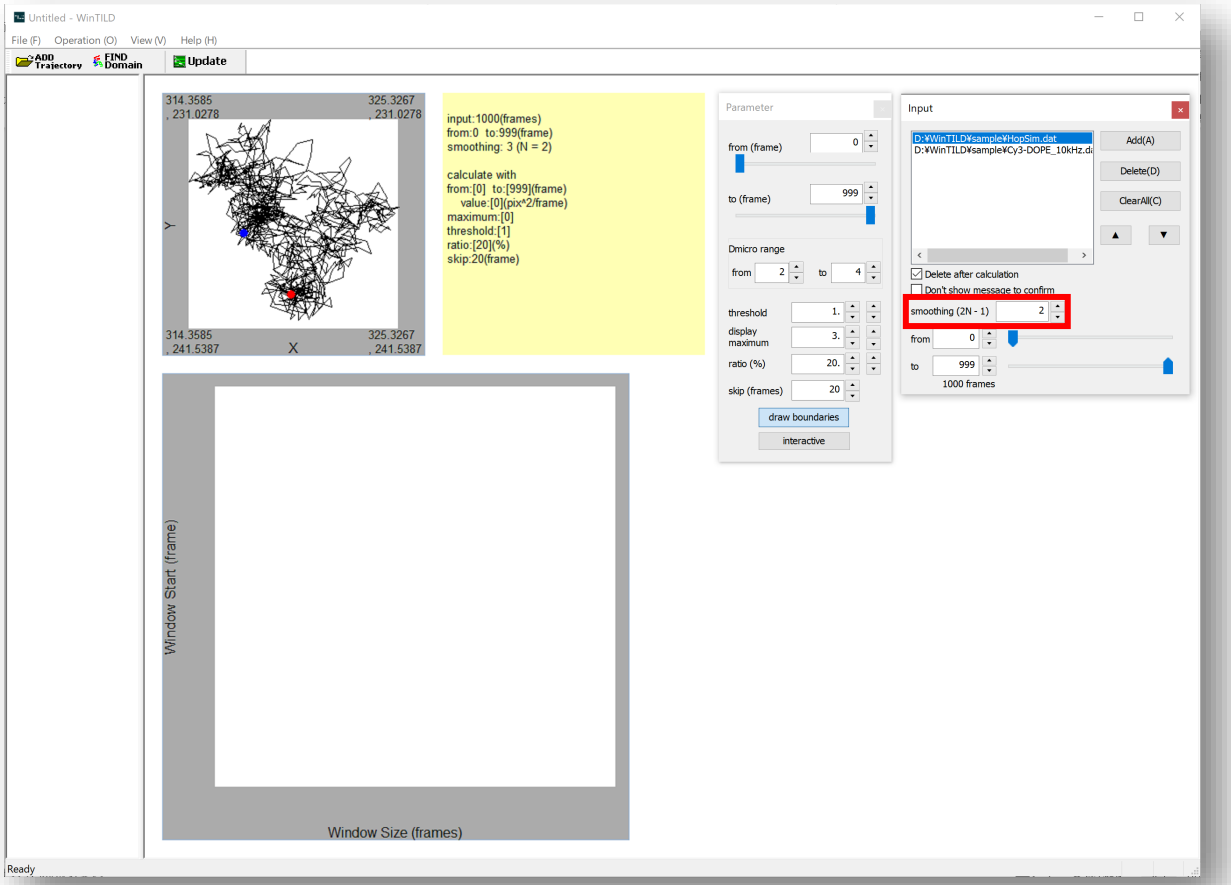
Load trajectories (1/3)

- To load single-molecule trajectories, click on “ADD Trajectory” toolbar icon to show “Input” panel. See the last page of this manual for the txt file format.
- Load trajectories (e.g. /sample/HopSim.dat and Cy3-DOPE_10kHz.dat) from the dialogue shown by “Add” button or directly drag-and-drop the files into the blank list area on the left. The trajectories are displayed by clicking on the filenames.



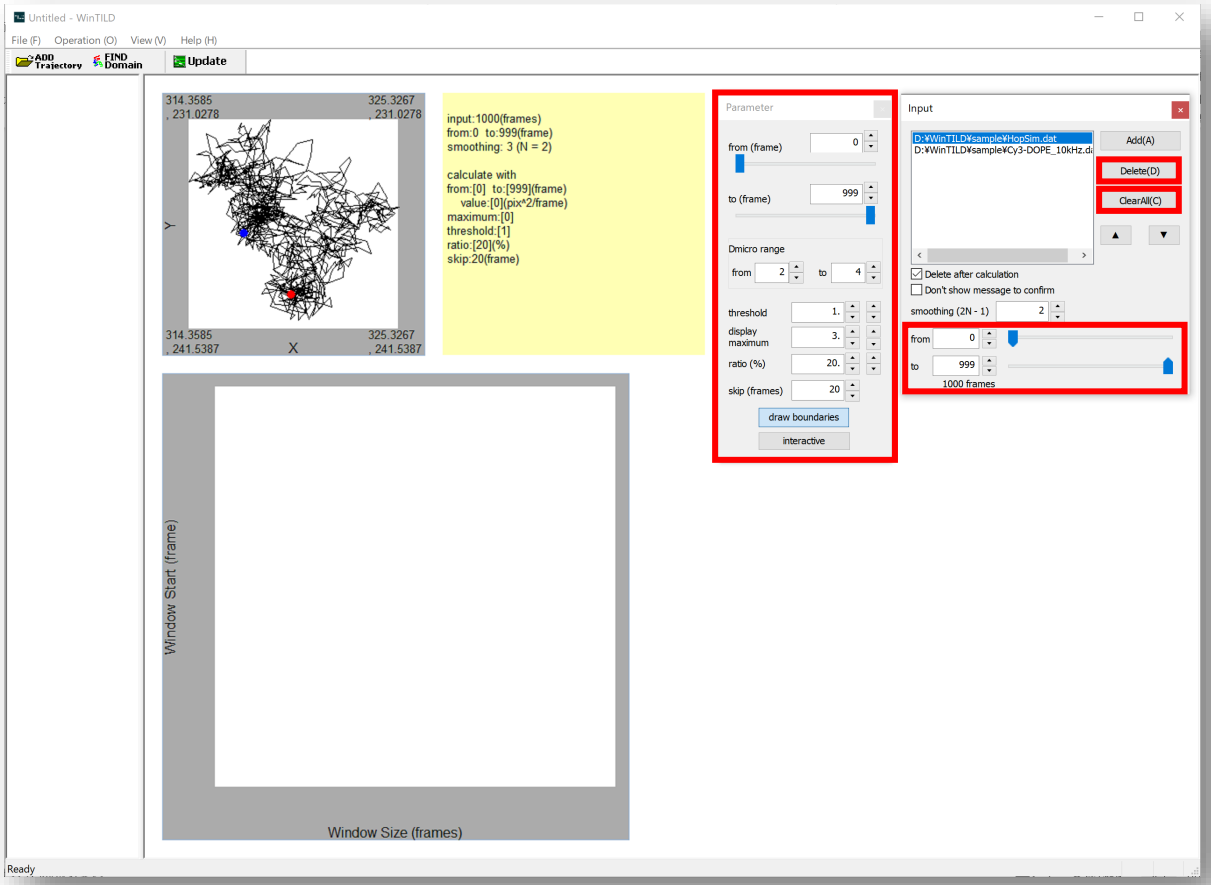
Load trajectories (2/3)

- To apply (2N-1)-frame running averaging to minimize the effect of apparently large displacements that stochastically occur when single-molecule localization errors are large, put the value of N into the "smoothing (2N-1)" box. When N = 2 is put, 3-frame running averaging (replacing the position of the kth frame with the position averaged for the k-1, k, and k+1 frames) is applied to all the trajectories in the list.

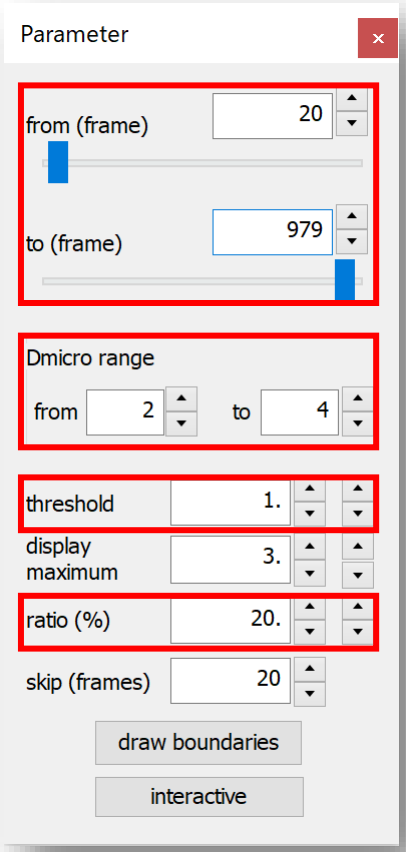


Load trajectories (3/3)

- The range of frames to be analyzed can be specified by the “from” and “to” boxes or the sliders on the right.
- The trajectory files in the list can be deleted by the “Delete” button or totally cleared by the “ClearAll” button.
- Specify the parameters in the “Parameter” panel before starting analysis.

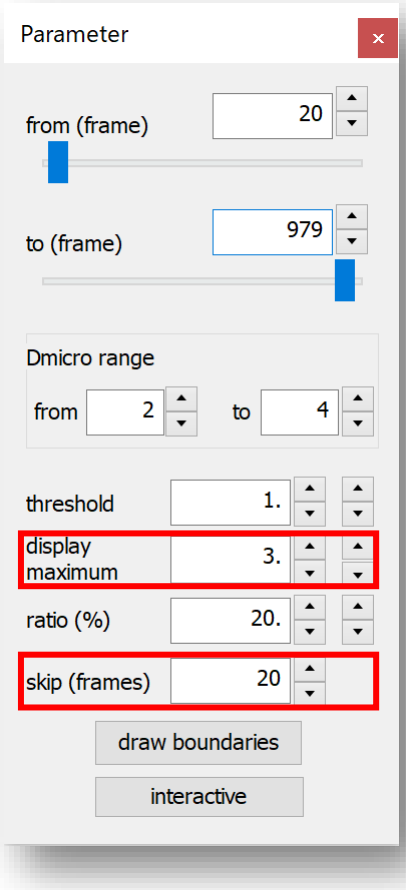


Set parameters (1/2)



- "from" and "to" set the range of Window Size (n; horizontal axis of the grayscale heatmap display) to be evaluated for TILD occurrence. In this example, 960 (20 – 979) window sizes are evaluated for each Window Start frame (m; vertical axis of the display) when the total length of each trajectory (N) is 1,000.
- "Dmirco range" sets the start (from) step and the end (to) step of the time interval for the short-term diffusion coefficient (default: $D_{2\text{-}4}$) used for normalizing the relative diffusion coefficient (D_{rel}).
- When the percentage of window sizes in which $H(m,n) \geq$ "threshold" value (default: 1) is greater than or equal to "ratio (%)" valule (default: 20) among total window sizes (960 in this example), the frame (m) is detected as a TILD moment.

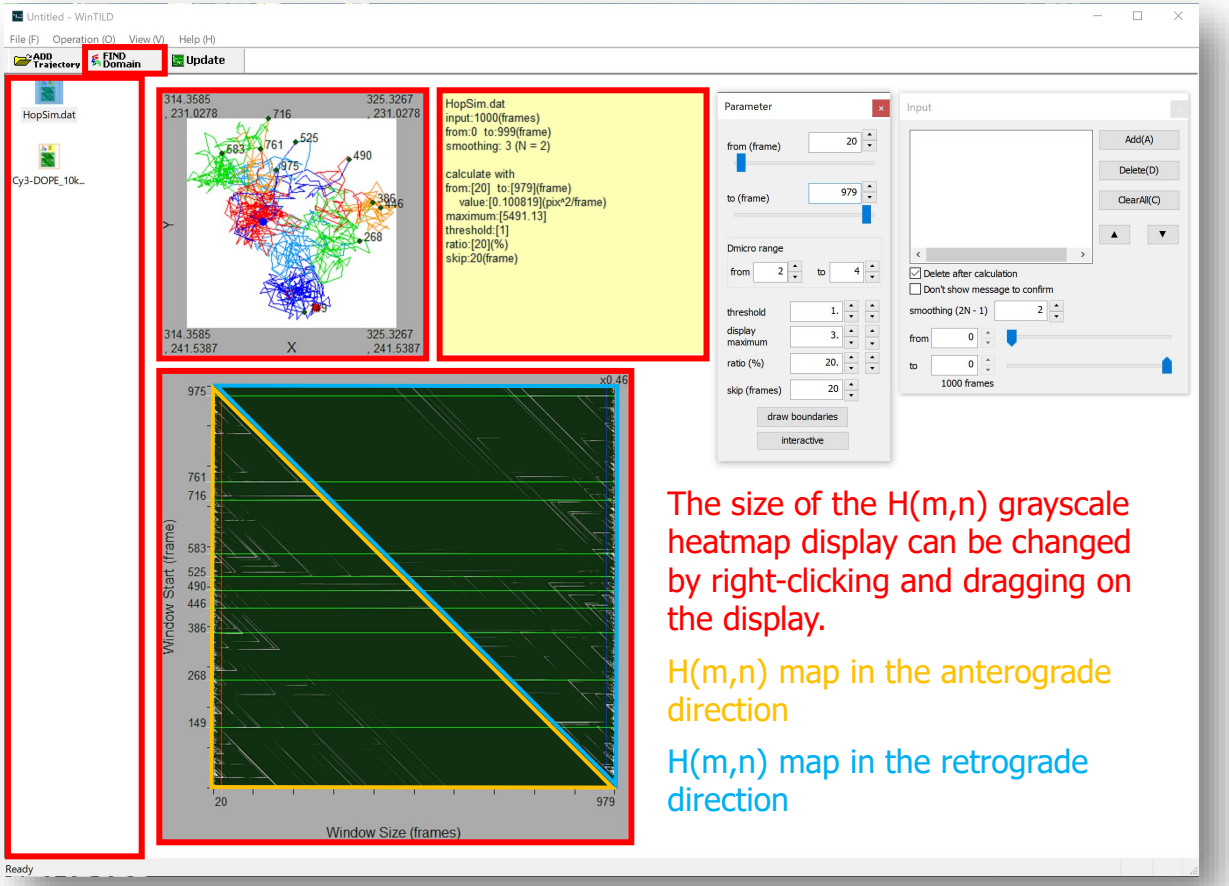
Set parameters (2/2)



- If a new TILD moment is detected within “skip (frames)” (default: 20) from the previous TILD moment, the new moment is neglected.
- “display maximum” sets the H(m,n) value for the maximum intensity (255) in the grayscale (0 – 255) heatmap display (default: 3).

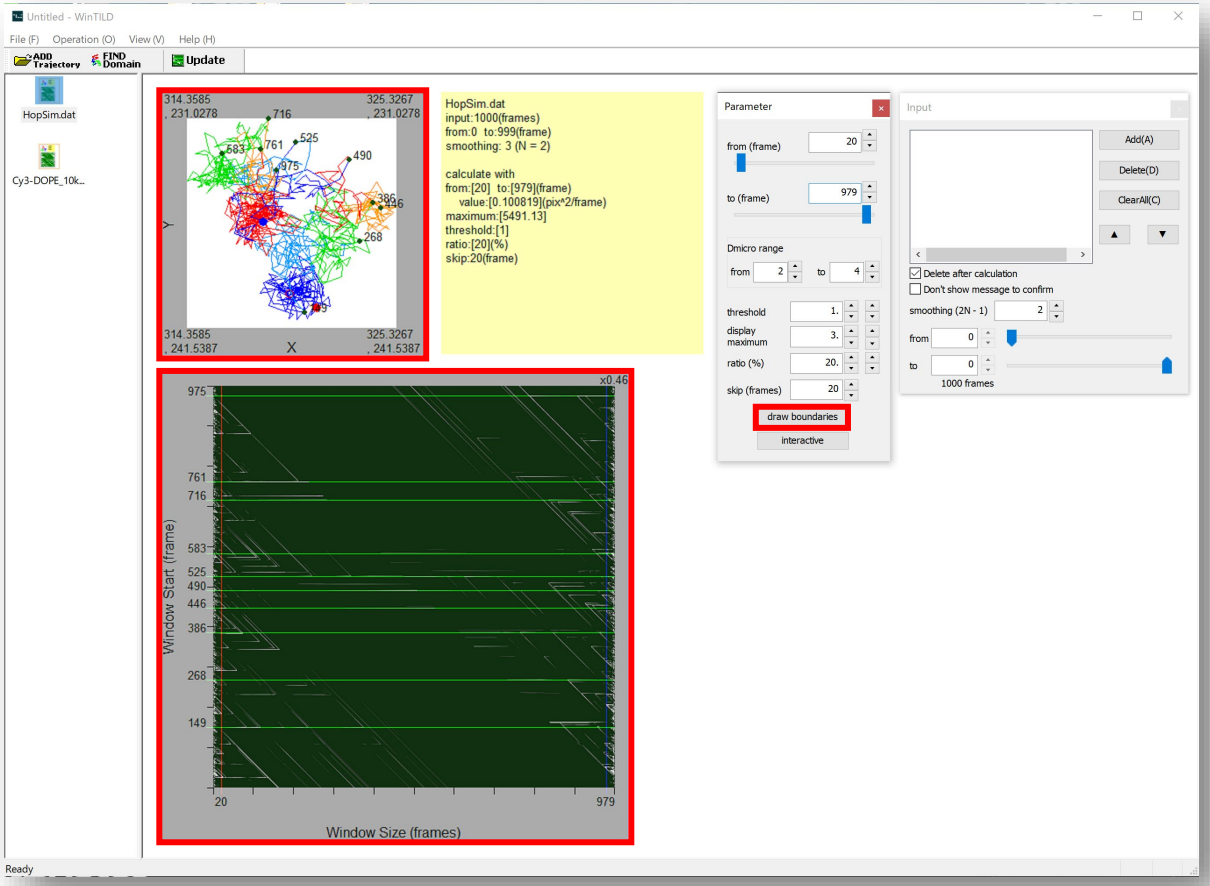
Run analysis and check results (1/4)

- Select trajectories to be analyzed in the list of the “Input” panel and start TILD analysis by clicking on “FIND Domain” toolbar icon.
- The results of individual trajectories are listed by the icons in the left pane. Clicking on each icon shows its color-coded trajectory, analysis parameters, and grayscale heatmap display of H(m,n).



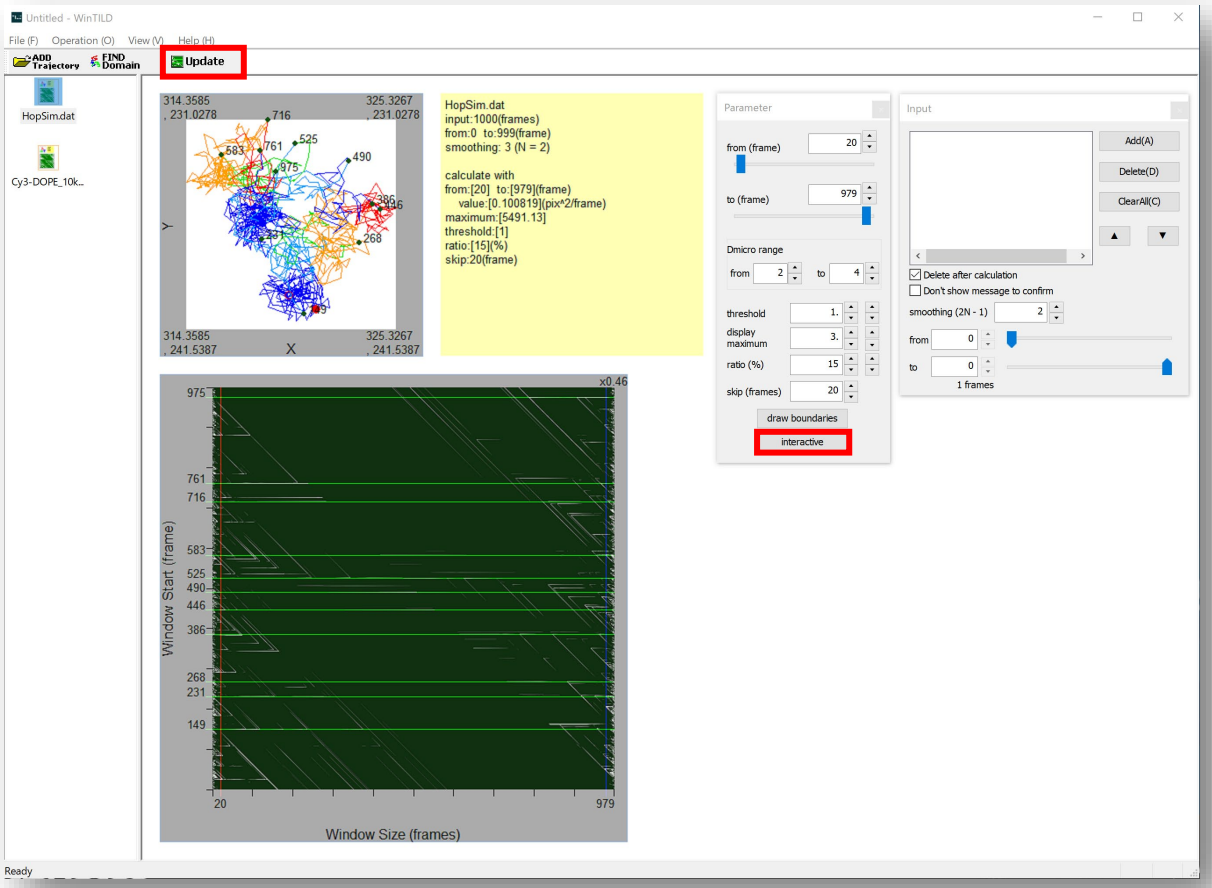
Run analysis and check results (2/4)

- The color-coding of the trajectory changes at each TILD moment in the order of blue, cyan, green, orange, red, and then back to blue.
- The frame numbers of detected TILD moments are shown on the vertical axis with green lines in the heatmap display. The lines toggle on and off by clicking on “draw boundaries” button of the “Parameter” panel.



Run analysis and check results (3/4)

- To perform re-analysis after changing parameters, click on the “Update” toolbar button.
- If “interactive” button on the “Parameter” panel is activated, the changes in parameters are immediately reflected in the analysis results.

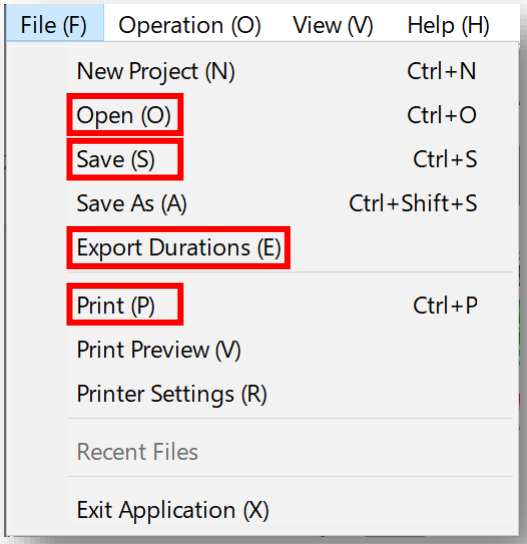


Run analysis and check results (4/4)

- Trajectory Viewer can be launched from “View > Play Dialog” menu. In the viewer, a color-coded dot indicating the current frame moves along the trajectory by clicking on play forward (|>) or play backward (<|) buttons with the specified frame rate (fps).

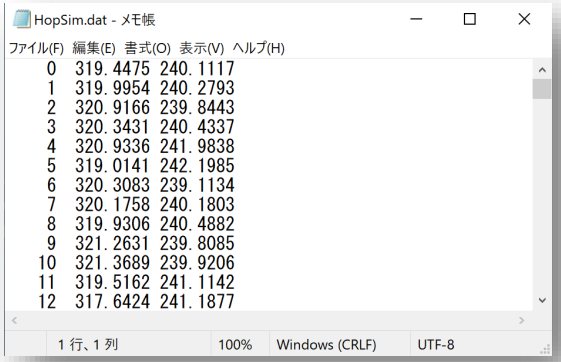
Save the project and export results

- The whole analysis results can be saved into the project file (*.dfp) by "File > Save" menu, and can be loaded again by "File > Open" menu.
- The frame numbers and XY positions of detected TILD moments and the duration frames between TILD moments can be exported into the txt file by "File > Export Durations" menu. See the last page of this manual for the format.
- The trajectory, parameter, and grayscale heatmap displays can be printed by "File > Print" menu. To export the displays into PDF, use "Microsoft Print to PDF" driver. "Adobe PDF" driver may not work properly with this software.



Data format

Input trajectory file


HopSim.dat - メモ帳

ファイル(F) 編集(E) 書式(O) 表示(V) ヘルプ(H)

0	319.4475	240.1117
1	319.9954	240.2793
2	320.9166	239.8443
3	320.3431	240.4337
4	320.9336	241.9838
5	319.0141	242.1985
6	320.3083	239.1134
7	320.1758	240.1803
8	319.9306	240.4882
9	321.2631	239.8085
10	321.3689	239.9206
11	319.5162	241.1142
12	317.6424	241.1877

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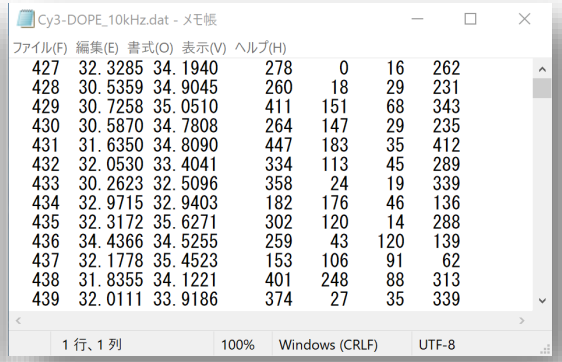
Cy3-DOPE_10kHz.dat - メモ帳

ファイル(F) 編集(E) 書式(O) 表示(V) ヘルプ(H)

427	32.3285	34.1940	278	0	16	262
428	30.5359	34.9045	260	18	29	231
429	30.7258	35.0510	411	151	68	343
430	30.5870	34.7808	264	147	29	235
431	31.6350	34.8090	447	183	35	412
432	32.0530	33.4041	334	113	45	289
433	30.2623	32.5096	358	24	19	339
434	32.9715	32.9403	182	176	46	136
435	32.3172	35.6271	302	120	14	288
436	34.4366	34.5255	259	43	120	139
437	32.1778	35.4523	153	106	91	62
438	31.8355	34.1221	401	248	88	313
439	32.0111	33.9186	374	27	35	339

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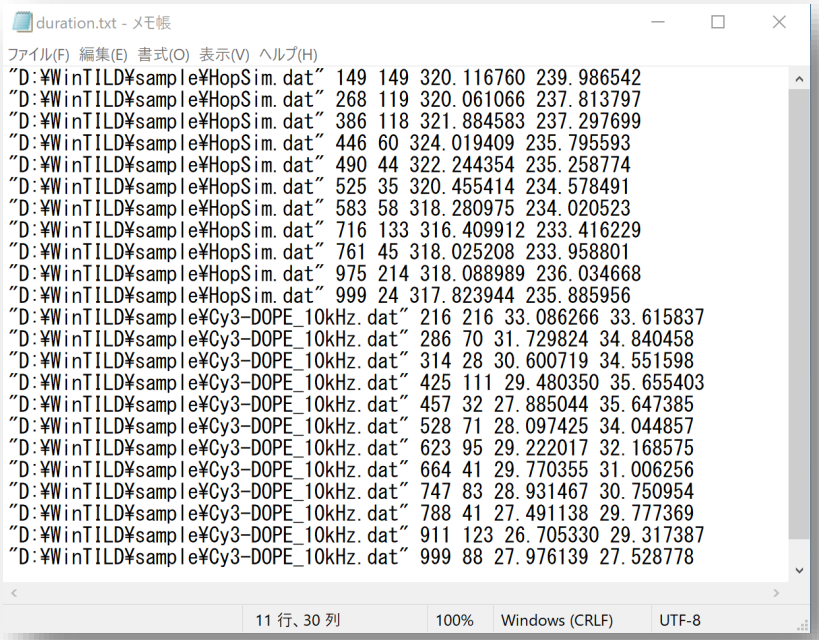
- Space or Tab-delimited txt file
- [frame X_pixel Y_pixel]
- The program skips loading the numbers following Y coordinates.
- The first frame number of the trajectory is set to 0 in the program.

Output duration file


duration.txt - メモ帳

ファイル(F) 編集(E) 書式(O) 表示(V) ヘルプ(H)

"D:¥WinTILD¥sample¥HopSim.dat"	149	149	320.116760	239.986542	
"D:¥WinTILD¥sample¥HopSim.dat"	268	119	320.061066	237.813797	
"D:¥WinTILD¥sample¥HopSim.dat"	386	118	321.884583	237.297699	
"D:¥WinTILD¥sample¥HopSim.dat"	446	60	324.019409	235.795593	
"D:¥WinTILD¥sample¥HopSim.dat"	490	44	322.244354	235.258774	
"D:¥WinTILD¥sample¥HopSim.dat"	525	35	320.455414	234.578491	
"D:¥WinTILD¥sample¥HopSim.dat"	583	58	318.280975	234.020523	
"D:¥WinTILD¥sample¥HopSim.dat"	716	133	316.409912	233.416229	
"D:¥WinTILD¥sample¥HopSim.dat"	761	45	318.025208	233.958801	
"D:¥WinTILD¥sample¥HopSim.dat"	975	214	318.088989	236.034668	
"D:¥WinTILD¥sample¥HopSim.dat"	999	24	317.823944	235.885956	
"D:¥WinTILD¥sample¥Cy3-DOPE_10kHz.dat"	216	216	33.086266	33.615837	
"D:¥WinTILD¥sample¥Cy3-DOPE_10kHz.dat"	286	70	31.729824	34.840458	
"D:¥WinTILD¥sample¥Cy3-DOPE_10kHz.dat"	314	28	30.600719	34.551598	
"D:¥WinTILD¥sample¥Cy3-DOPE_10kHz.dat"	425	111	29.480350	35.655403	
"D:¥WinTILD¥sample¥Cy3-DOPE_10kHz.dat"	457	32	27.885044	35.647385	
"D:¥WinTILD¥sample¥Cy3-DOPE_10kHz.dat"	528	71	28.097425	34.044857	
"D:¥WinTILD¥sample¥Cy3-DOPE_10kHz.dat"	623	95	29.222017	32.168575	
"D:¥WinTILD¥sample¥Cy3-DOPE_10kHz.dat"	664	41	29.770355	31.006256	
"D:¥WinTILD¥sample¥Cy3-DOPE_10kHz.dat"	747	83	28.931467	30.750954	
"D:¥WinTILD¥sample¥Cy3-DOPE_10kHz.dat"	788	41	27.491138	29.777369	
"D:¥WinTILD¥sample¥Cy3-DOPE_10kHz.dat"	911	123	26.705330	29.317387	
"D:¥WinTILD¥sample¥Cy3-DOPE_10kHz.dat"	999	88	27.976139	27.528778	

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- Space-delimited txt file
- [trajectory TILD_frame duration_frames X_pixel Y_pixel]