WinTILD Quick Manual

Reference

Development of ultrafast camera-based imaging of single fluorescent molecules and live-cell PALM

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Version 1.0

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.
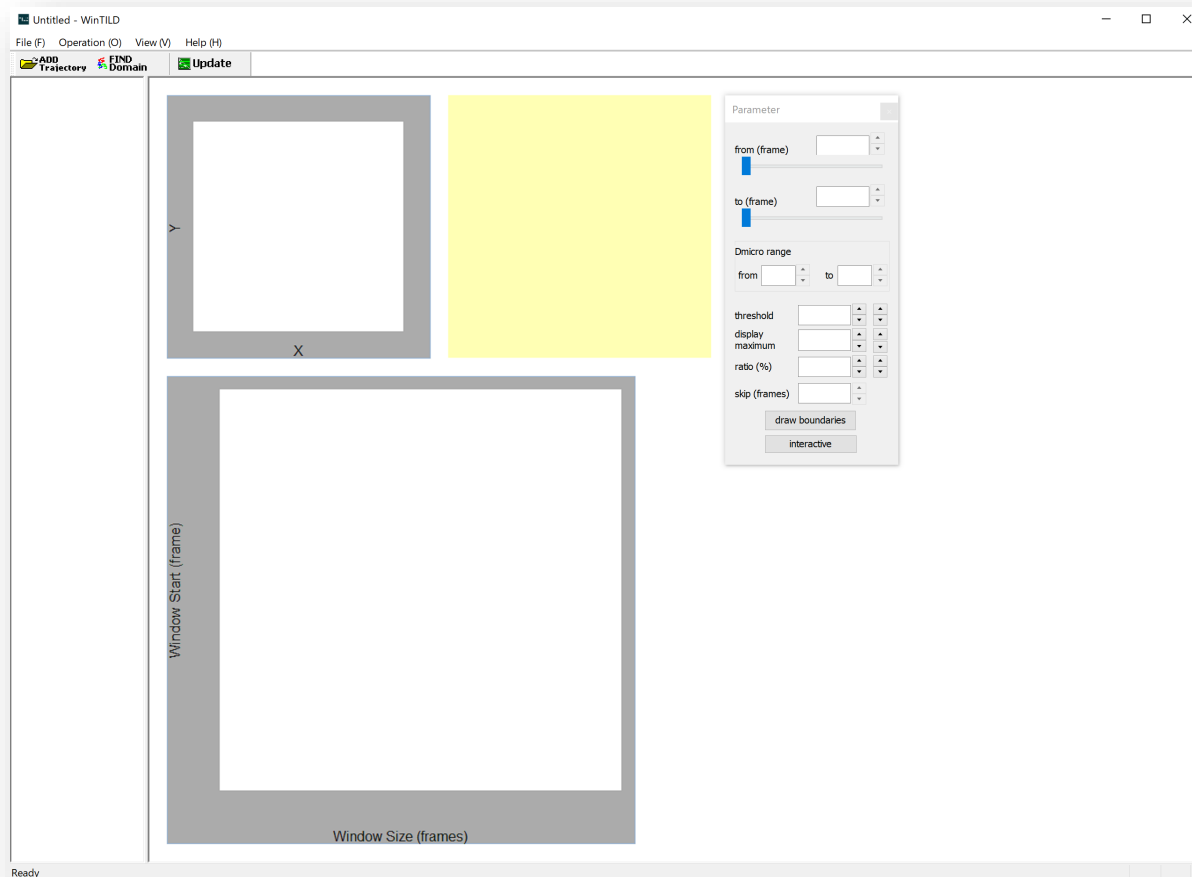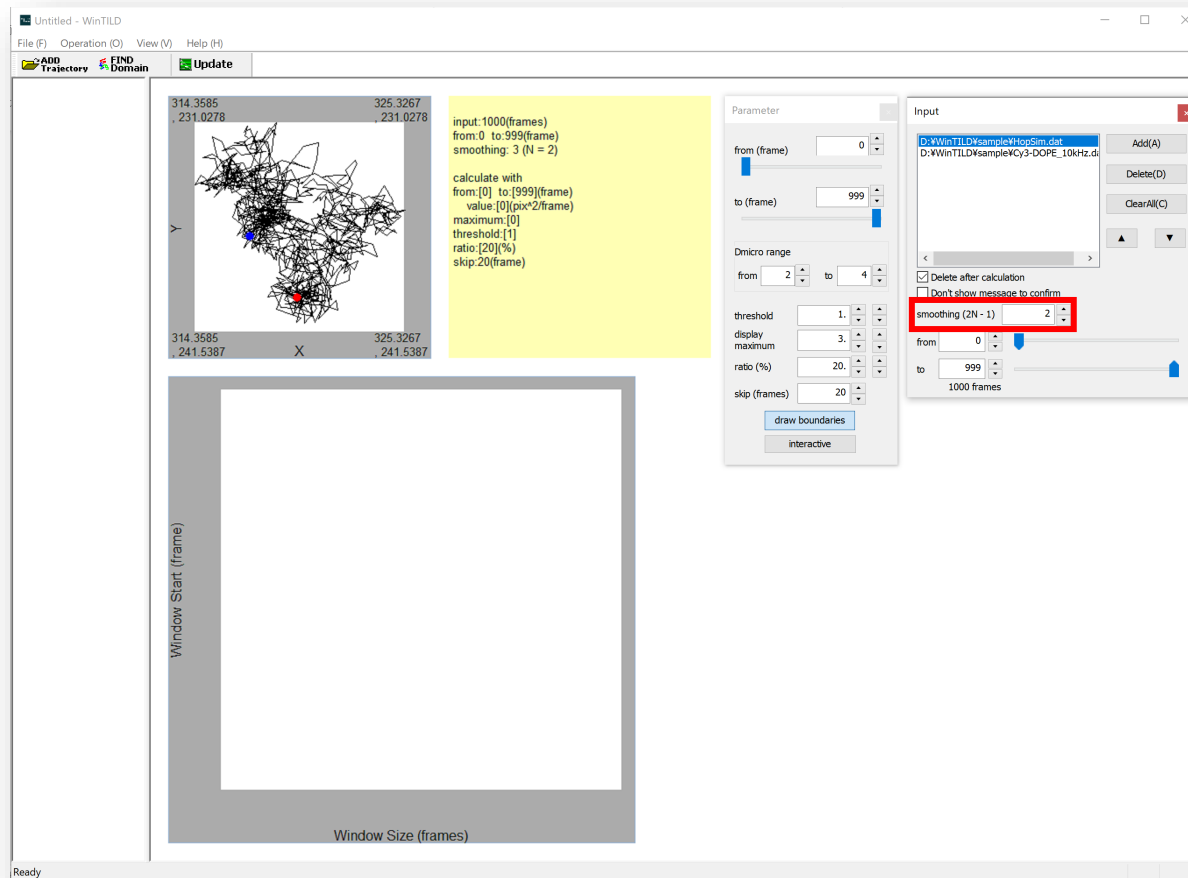
Load trajectories (1/3)

- 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.
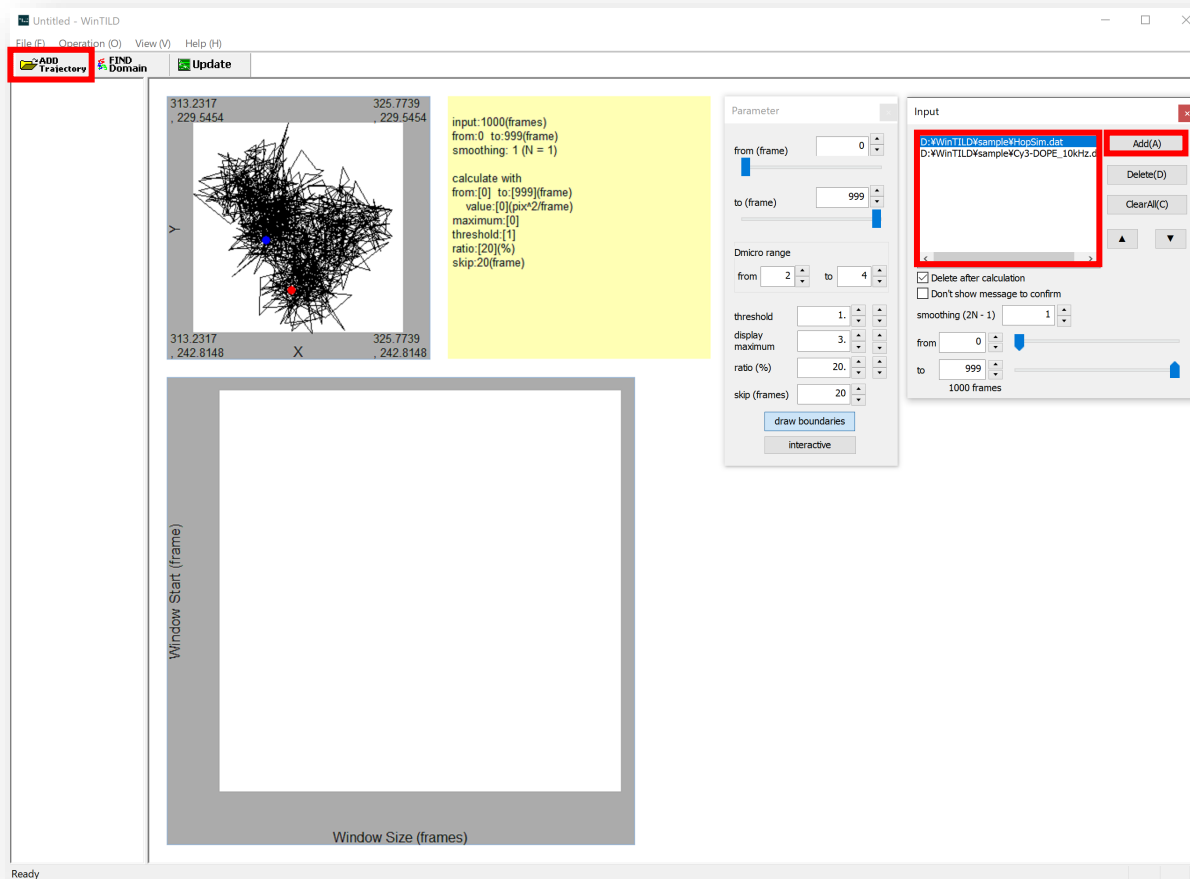
Load trajectories (2/3)

- 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.
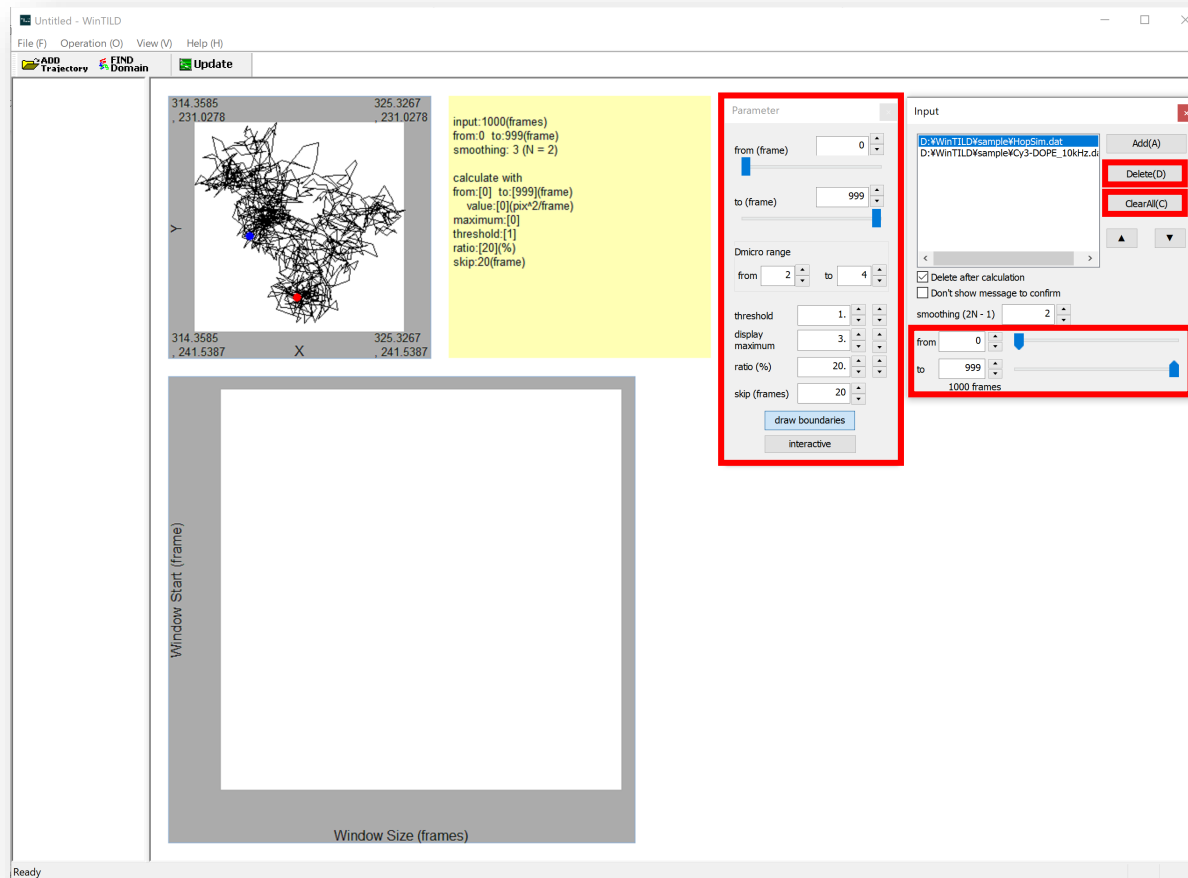
Set parameters (1/2)

- "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_{2-4}) used for normalizing the relative diffusion coefficient (D_{rel}).

- When the percentage of window sizes in which H(m,n) ≥ "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)
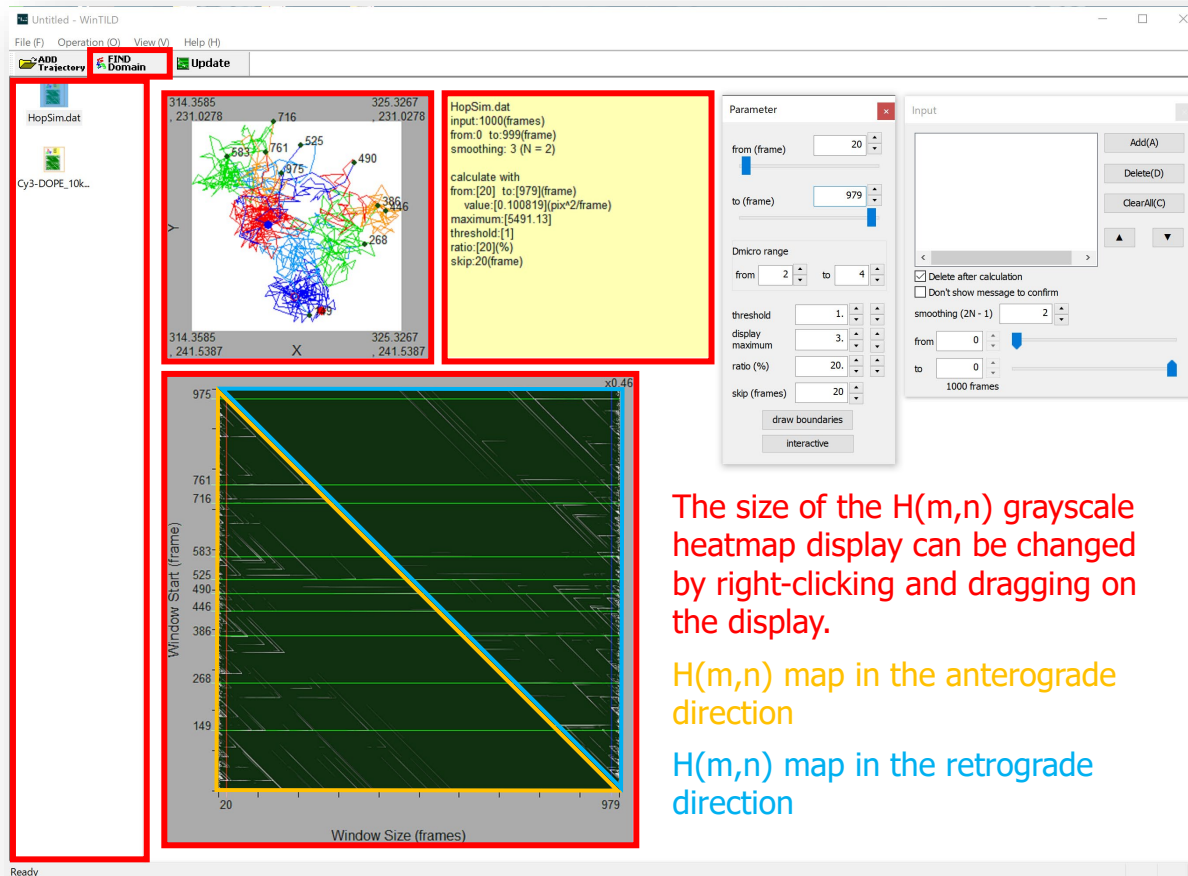

- If a new TILD moment is detected within "skip (frames)" (default: 20) from the previous TILD moment, the new moment is neglected.

- "display maximum" sets the $H(m,n)$ value for the maximum intensity (255) in the grayscale (0 – 255) heatmap display (default: 3).

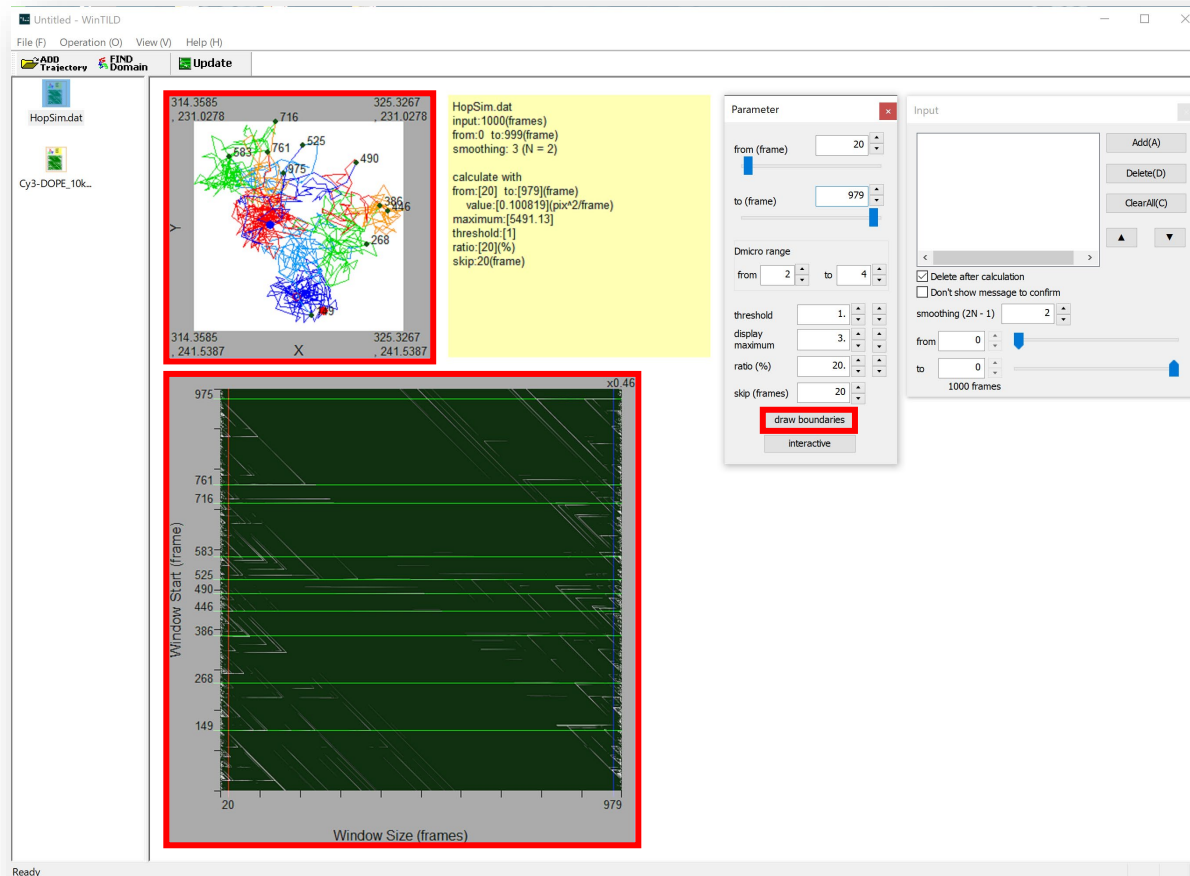# Run analysis and check results (1/4)

- 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).


The size of the H(m,n) grayscale heatmap display can be changed by right-clicking and dragging on the display.

H(m,n) map in the anterograde direction

H(m,n) map in the retrograde direction

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.
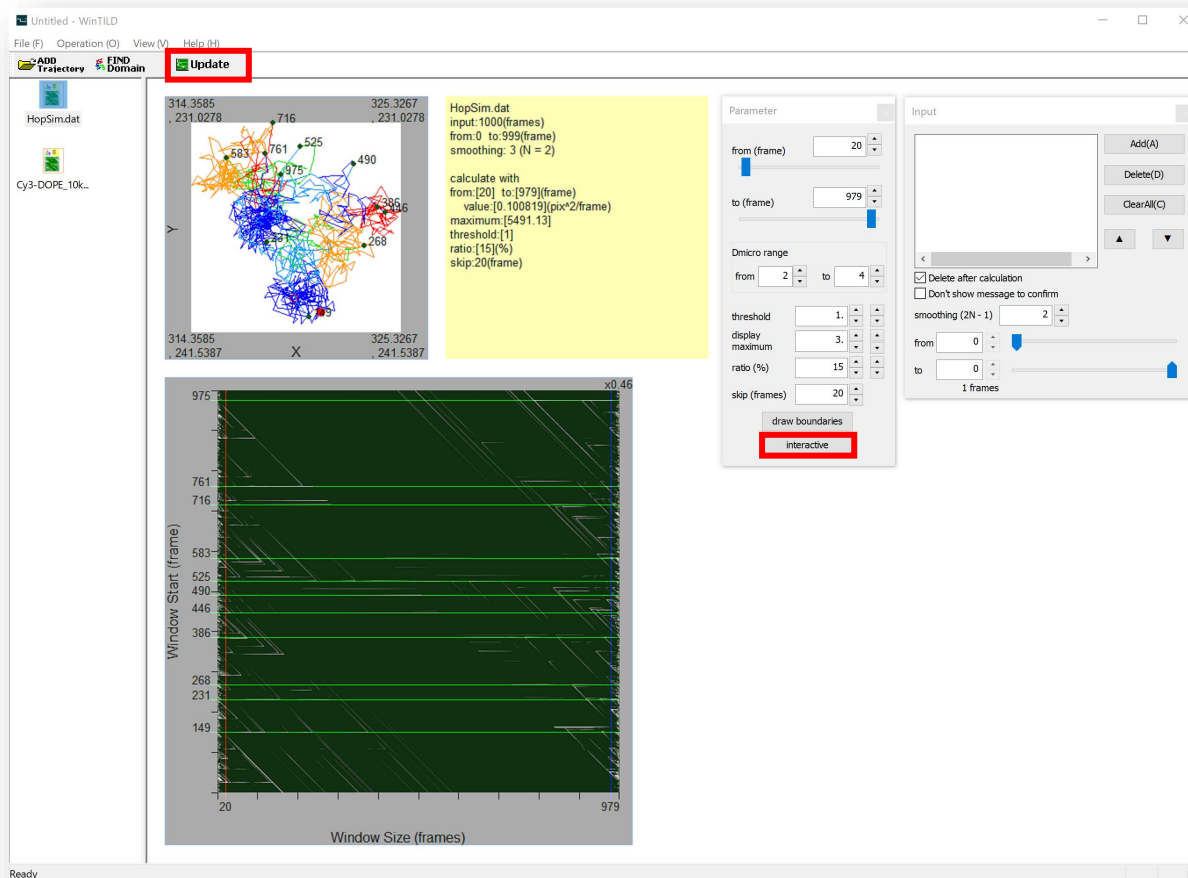
Run analysis and check results (4/4)

- 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).
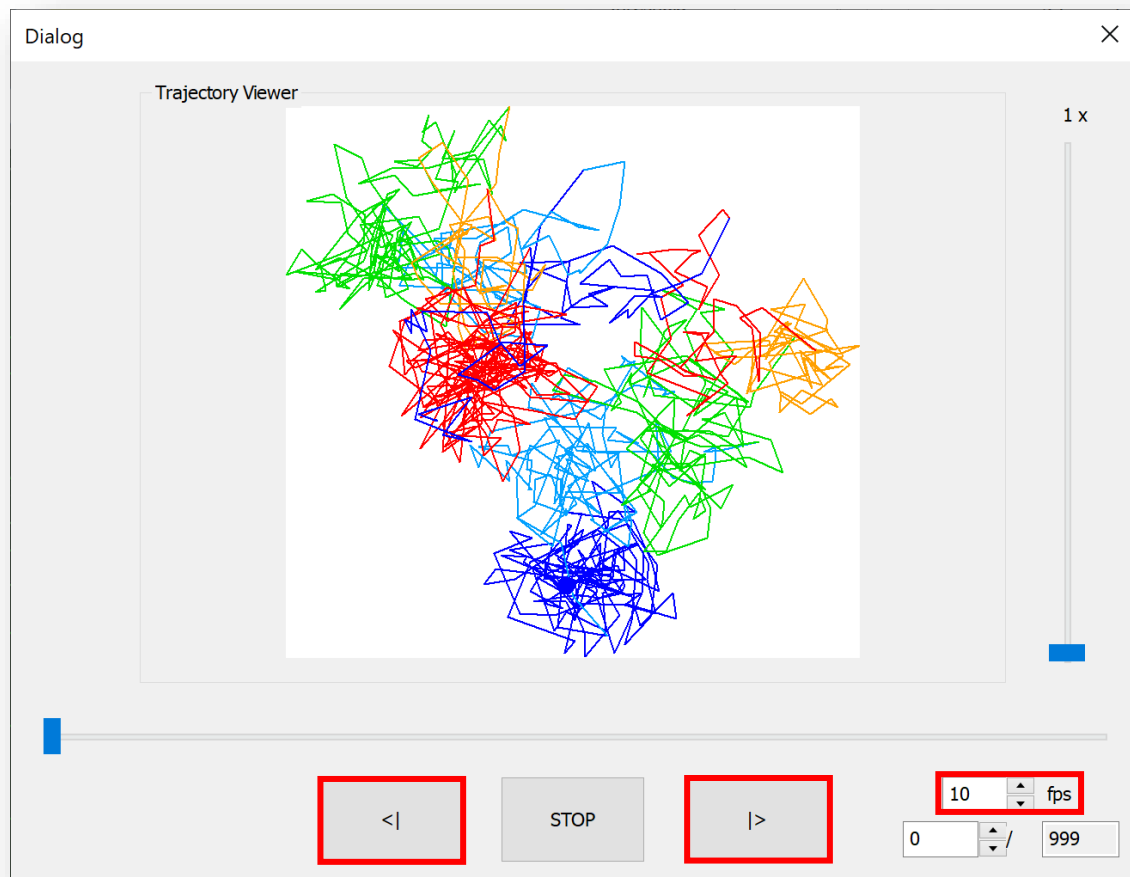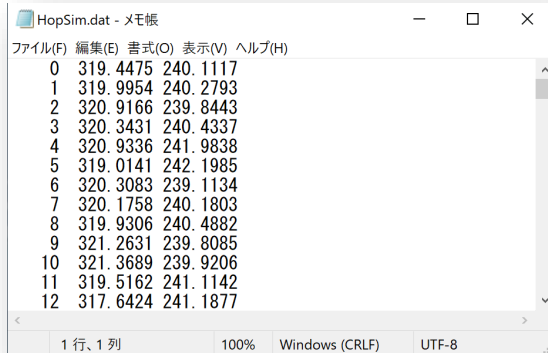
Save the project and export results

- 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.

File (F)	Operation (O)	View (V)	Help (H)
New Project (N)			Ctrl+N
Open (O)			Ctrl+O
Save (S)			Ctrl+S
Save As (A)			Ctrl+Shift+S
Export Durations (E)			
Print (P)			Ctrl+P
Print Preview (V)			
Printer Settings (R)			
Recent Files			
Exit Application (X)			
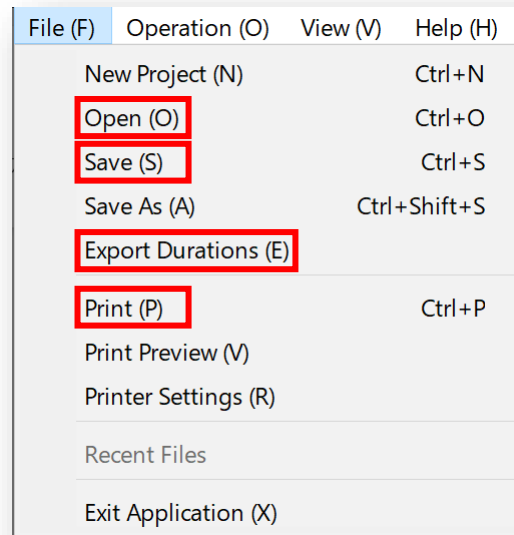
Data format

Input trajectory file

- 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

- Space-delimited txt file

- [trajectory TILD_frame duration_frames X_pixel Y_pixel]