

Single-molecule tracking data – ReadMe File

Together with this file comes two directories, “slowSPT_data” and “spaSPT_data”, containing all the raw trajectory data for the single-molecule tracking experiments. For details of how the experiments were performed and acquisition parameters, please see the description in the methods section of the manuscript.

Each folder contains a series of Matlab MAT files (CSV files are also provided in separate directory). More specifically, all the trajectories from each single cell are provided in a single CSV file and replicates provided as additional CSV files. The name of the CSV file describes the experiment (slowSPT or spaSPT), the protein of interest (e.g. Halo-CycT1_WT) and the frame rate (e.g. 2 Hz or 95 Hz).

Each MAT file contains 2 structured arrays:

- “settings”: which contains metadata from the experiment
- “trackedPar”: which contains the SPT data in the form of trajectories

More specifically, trackedPar contains three variables:

- trackedPar.xy: “xy” is a matrix with 2 columns and a number of rows corresponding to the number of localizations in that trajectory. The first column is the x-coordinate and the second column is the y-coordinate. The units are micrometers (μm).
- trackedPar.Frame: “Frame” is a column vector where each element is the frame where the particle was localized.
- trackedPar.TimeStamp: “TimeStamp” is a column vector where each element is the timepoint where the particle was localized.

Each element in the structure array “trackedPar” corresponds to a different trajectory.

In the case of the spaSPT data, the Matlab “trackedPar” format is readable by Spot-On. The Matlab version of Spot-On can be found on GitLab: <https://gitlab.com/tjian-darzacq-lab/spot-on-matlab>; Thus, if you download the Matlab version of Spot-On you can directly reproduce our results from the attached data.