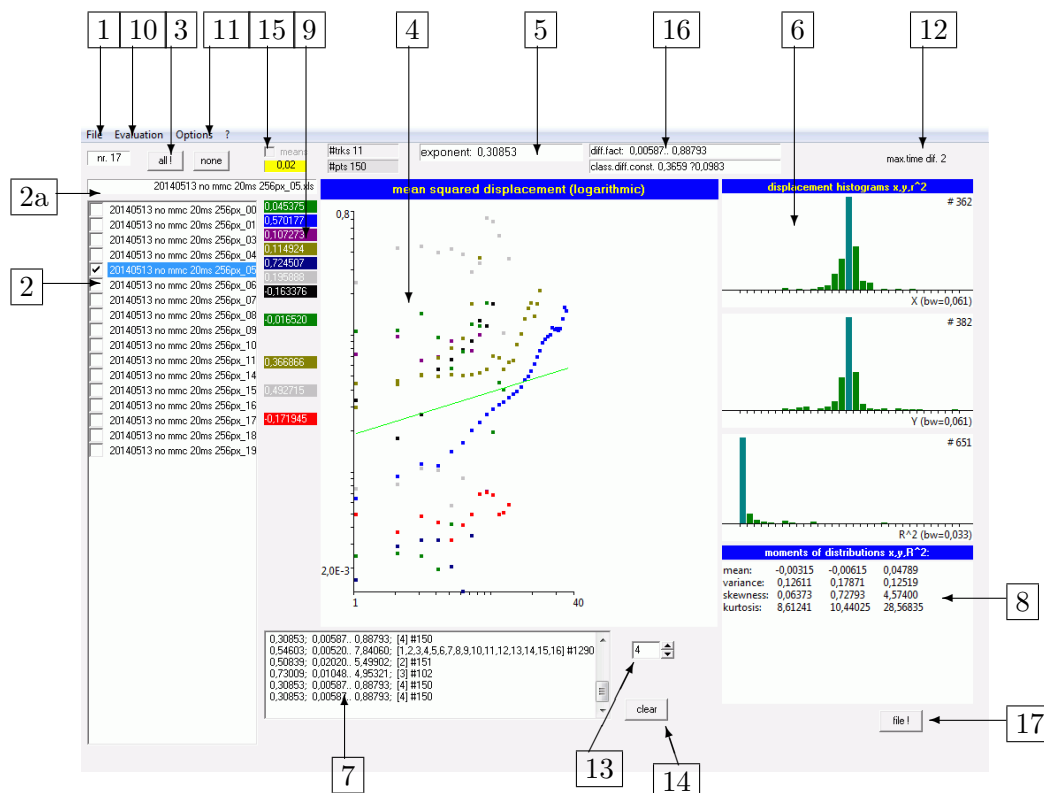


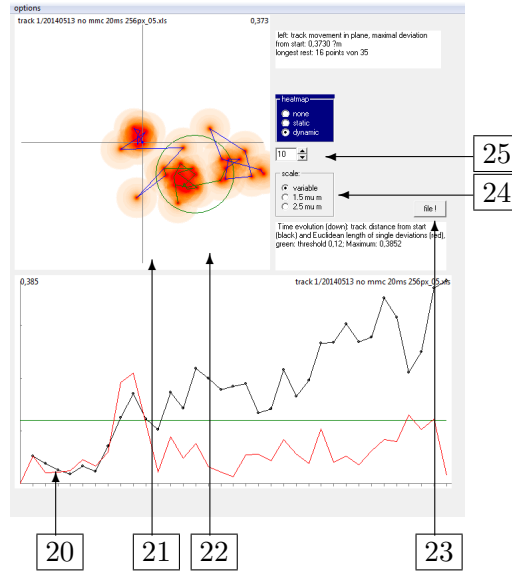
## SMMTrack user manual

The program **SMMTrack.exe** displays and analyzes **Tracking** data files from **Single-Molecule-Microscopy** observations. The following screen shot shows its main GUI window with pointers to its controls and displays.



Tracking data must be sampled with a fixed time cycle  $\tau$  and must be provided as pure text files with extensions '.xls' or '.csv'.

- Type '.xls' contains a one-line header for columns **Nr TID PID x[ $\mu\text{m}$ ] y[ $\mu\text{m}$ ] t[sec]**, etc. followed by lines of data with columns separated by blanks. Additional columns are ignored. Each file may contain several tracks distinguished by **TID**.
- Type '.csv' contains the same data with no header, data separated by comma.



Second GUI window displaying the movement of individual tracks. This window opens under the conditions described in [9].

The program file **SMMTrack.exe** should be situated in the directory above the data directory in order to facilitate file selection [1].

1. Menu *File*, item *read tracks (.xls)* or *read tracks (.csv)* opens a file dialog box for selection of a sub-directory. **SMMTrack** displays a list of all data files of the chosen type from this directory in window [2], and the path is displayed in the header of the GUI window.
2. The file list box [2] displays a check-list of these track data files. After checking individual files track data are included immediately in the analysis and displays. Since file names may be too long for the list box the most recently chosen file name is also displayed in [2a]. Files may also be unchecked again. The mean squared displacement (MSD) of all checked tracks appear in diagram [4], furthermore [6] displays the distributions of all single track increments in  $x$  and  $y$  directions and the squared Euclidean lengths. The first 4 moments of these distributions are shown in [8] in order to judge deviations from the normal distribution.

In order to allow analysis of single tracks an additional colored menu bar appears in [9]. The colors correspond to those used in the MSD diagram [4].

3. Buttons `[all]`, `[none]` select all or no files from `[2]`.
4. The box `[4]` displays a diagram of the mean square displacement (MSD) over time of all track files checked in `[2]`. Data from individual tracks are distinguished by 8 colors. Color assignment is identical with that from `[9]`. The  $MSD(k\tau)$  from one or several chosen tracks which depend on the cycle number  $k$  are displayed in double logarithmic scale. This leads to a linear behaviour  $\log MSD(k\tau) = \log \beta + \alpha(\log k)$  for functions of the form  $MSD(k\tau) = \beta \cdot k^\alpha$ . A linear regression of all used MSD data is displayed as a line in light green. However, since the constant  $\beta$  may vary extremely between different tracks, regression is based on an ansatz  $MSD_i(k\tau) = \beta_i \cdot k^\alpha$  for track nr. $i$  where only the exponent  $\alpha$  is a common parameter. The green line shown uses a mean  $\beta$ -value, see also `[5]`. The scale of the diagram is adjusted automatically but uses the same scaling in both directions by default ( $\rightarrow$  `[11]`, *MSD isotropic*).
5. The field shows the exponent  $\alpha$  described in `[4]` and the range of factors  $d_{i,1} = \beta_i/\tau^\alpha$  belong to the chosen tracks.
6. The 3 windows `[6]` display histograms of the track increments, i.e. the differences of consecutive track points. Increments in both space directions should be normally distributed. The third diagram shows squares of  $x$ - and  $y$ -increments where a  $\chi^2$ -distribution with 2 degrees of freedom ( $\sim e^{-x/b}$ ) is expected. Scales are adjusted automatically and the value `bw` gives the width of each bar in the histogram in the data region.  
  
For all 3 histograms `[8]` displays the estimates for the moments of their distribution. This window may be overwritten by other user actions.
7. A text log is shown in this window. With a white background results  $(\alpha, d)$  of the regression are shown for the selected data from `[2]` with a list of tracking files used (files denoted by their place in the file list). If one single track is selected in `[9]` positions relative to the starting point are displayed here with a grey background.

8. Window [8] has different purposes. After changes in track selection it shows the moments of the distributions shown in [6]. However, also diagrams called from the main menu under *Evaluations* [10] are displayed here.
9. This menu of colored bars for individual tracks is displayed if only one single track file is checked in [2]. The color of each box corresponds to the color in the MSD diagram [4]. The number in each box is its individual sub-diffusion exponent  $\alpha$ . There may be gaps in the menu corresponding to single tracks too short for evaluation. If one colored bar is selected MSD data are shown for this track only in [4] and its data in [7] for inspection. Each line in [7] gives the time-,  $x$ -, and  $y$ -difference from the first track point.

If the item *show track diagrams* has been checked under the *Options* menu [11], choosing one colored bar also opens a second window [20] displaying its 2D-trajectory and an optional heatmap.

10. Menu item 'Evaluations':
  - *'track lengths'*: shows a histogram of the lengths of all tracks in [8]. One may notice here if short tracks have been masked.
  - *'track radii'*: shows a histogram of the maximal Euclidean distance of each track from its start. This item may help to detect space restrictions for the track (e.g. through cell walls) which may lead to flattening of MSD diagrams without subdiffusion.
  - *'resting times'*: shows a histogram of periods of times of rest within tracks in [8]. Resting is defined as a movement which does not leave a threshold radius determined by the optical resolution  $120nm$ . Times of rest are computed by checking forward and backward in time from each track point how long the track remains within the threshold radius. The longest such time interval is counted as a time of rest. Then, all track points belonging to this longest interval are removed from the shorter resting intervals for the sake of statistical independence. Then, the process is repeated with the second longest interval and so on. Data for the longest interval of rest are saved and displayed

in the 2D-track diagram [22] as a green circle with the threshold radius. Tracks with gaps are not included. The numerical values of this histogram are saved in the file ('-resting.txt') in the data directory.

- *'Increments k'*,  $k = 1..6$ : As a different way to detect resting within tracks a histogram is generated for the maximal distance between two track points within  $k$  time cycles with a cut-off for larger distances. In contrast to the previous item resting times longer than  $k$  may be counted multiply. Tracks with gaps are not included. Peaks in the distribution at small radii may indicate repeated short resting. This analysis has the advantage that it does not rely on knowledge of the threshold radius. Data are written to the file '-pause1..6.txt'.
- *'heatmaps'*: Here, a series of large bitmap graphics files 'heatmp??.bmp' is written with the heatmaps of all selected tracks, see [22]. Each file contains up to 100 heatmaps which are identified by a header in each heatmap and files are numbered with 2 digits.

11. 'Options': Items may be checked or unchecked for the following effects.

- *show track diagrams*: Only if this item is checked selecting a colored track bar in menu [9] will open the second window with the track diagrams  $\rightarrow$  [20].
- *MSD isotropic*: If item is checked (default) the MSD diagram [4] uses equal scaling in the horizontal and vertical direction. Then, the exponent  $\alpha = 1$  corresponding to ordinary diffusion has slope one but the diagram may use only part of the window.
- *MSD real time*: the horizontal axis in diagram [4] does not show the number of time cycles but the true time (in [sec]).
- *logfile on*: Any change in the file selection in [2] writes to the following log files. The files '-logfile.txt' (vgl. [17]), "-hist-x\*.txt", "-hist-Rq.txt" (histogram data of single increments) and "-increments.txt" (list of increments) are generated.

12. The box shows the maximal gap in the track counted in multiples of the cycle time. Tracks with gaps are excluded in some computations.

13. Dial for the minimal length of tracks (between 2 and 10). Tracks containing less points will be ignored, diagrams are updated immediately.
14. Button clears the text log box 7.
15. The box displays the common time cycle  $\tau$  for all track data in seconds. This value is extracted from the first file name if it contains a time scale in the Form "...ms". Otherwise, the difference of the first 2 times read from file is used.
16. The text boxes show the factors mentioned in 5  $d_{i,1} = \beta_i/\tau^\alpha$ . Also, the classical diffusion constant belonging to Brownian motion is shown,  $D = MSD(\tau)/(4\tau)$  with error interval, i.e. the standard deviation of increments relative to the time cycle  $\tau$ .
17. With the button file ! all 5 diagrams from the main window are written to bitmap graphics files in the data sub-directory. Also the text file 'logfile.txt' is written with a list of the data used and results of their analysis.
20. **Second GUI window for track diagrams** opened by 9. The lower diagram shows the time behavior (horizontal axis: time cycles) of the Euclidean distance of one selected track from its starting point (black) and the Euclidean lengths of single increments (red). The level of the threshold radius of the optical resolution is shown as a green horizontal line. If the green line is not visible the track remains below the threshold radius and may be considered as immobile. Track identifier and exact scale are given at the top of the diagram.
21. In the upper part of the window a quadratic region displays the 2D-movement of the track itself with its starting point in the middle, track-id and scale on the top again. If the resting times ( $\rightarrow$  10) have been computed before, the longest time interval of resting is painted green and the corresponding circle with the threshold radius is drawn.
22. For longer tracks it may be difficult to see where they rest or cross. For better visualization a heatmap may be generated as overlay. For

this map each track point is assumed to generate some amount of heat in its neighborhood and summing these bell shaped heat sources results in darker red color in areas where the track moves slowly or not at all. However, this description generates a *static heatmap* which does not distinguish between resting and crossing over. Hence, a *dynamic heatmap* is shown by default where cooling applies after the molecule has moved away. The dynamic heatmap shows the maximal temperature at any point and may be able to distinguish between real resting events and crossing. The scale may be chosen automatically or fixed at  $1.5\mu\text{m}$  or  $2.5\mu\text{m}$ , see [24].

23. With the button **file !** bitmap files with these diagrams are written in the data directory as '-D1track.bmp' and '-D2track.bmp'. Also, for printing, a high-resolution version of the 2D-diagram [22] is written to the bitmap file '-D2trackh.bmp'. For better visibility of the track in the large bitmap the option *thick lines* should be checked in the options menu.
24. Since it is difficult with automatic scaling to compare different heatmaps the default is a scale of  $1.5\mu\text{m}$  from the origin. Automatic scaling or a scale of  $2.5\mu\text{m}$  (length of a cell) may be chosen here.
25. Proper coloring of the heatmap may be difficult. This dial adjusts the rate of decay of the bell-shaped heat mask.

*Note:* All text and bitmap files are generated with default names only depending on the purpose and may be overwritten by the next user actions. Conservation of those files requires renaming. Also, bitmap files are very large and should be converted to compressed file formats like PNG.

When using this software **SMMTrack** please cite the paper *K. Schenk, M. Eisemann, A.B. Hervas, B.A. Schmitt, S. Dahlke, L. Kleine-Borgmann, S.M. Murray, P. Graumann: Rapid turnover of DnaA at replication origin regions contributes to initiation control of DNA replication, PLOS Genetics, 2017.*