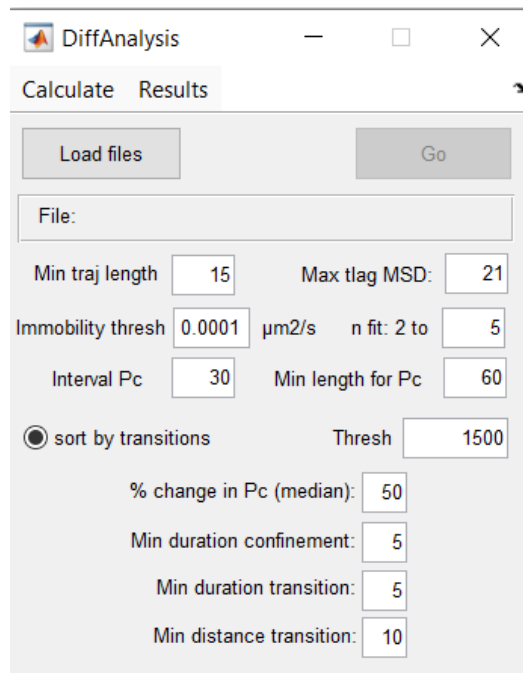# Diffusion analysis:

Please cite as:
Renner M, Wang L, Levi S, Hennekinne L, Triller A. (2017) A Simple and Powerful Analysis of Lateral Subdiffusion Using Single Particle Tracking. Biophys J. 113:2452-2463.

For each trajectory selected with **Load files**, this program will calculate (if the trajectory has at least **Min traj length** points):

- MSD and D for the whole trajectory or, if there the trajectories were localized, the MSD and D for each segment with different localization ("in" or "out" domains determined by the localization image). From the MSD calculation, it will save the first **Max tlag MSD** points of the MSD. Note: the MSD is calculated over the whole trajectory, but only the number of frames indicated here is kept for the summary files. This is helpful in the case of long trajectories, to speed up the calculation. D is calculated fitting MSD values from tlag=2 to the value entered in "**n fit: 2 to**". When compiling results, the threshold for immobility (**Immobility thresh**) is used to discard D and MSD values for trajectories that are defined as "immobile" in this way. This threshold corresponds to the diffusion measured on immobile tracers, and it is never zero due to the limited pointing accuracy.

- the packing coefficient (Pc) of the trajectory over a sliding window of **Interval Pc** frames, if the trajectory has a minimum of length (**Min length for Pc**).

- the dwell time.

- it will sort trajectories if **sort by transition** is chosen, using the Pc threshold **Thresh** (above vs below this value).

To avoid spurious classification, trajectories are considered as displaying transitions if the Pc values are above the threshold during **Min duration confinement** frames, with a change in Pc values above **% change in Pc**, with a transition that lasted at least **Min duration transition** frames, and a minimum distance between transition of **Min distance transition** (in nm).

Go: starts the analysis. The calculation of MSD, D and Pc are done by default. The program saves all in a .tnd file (one per movie), that can be read only with MatLab.

If you select "**Sort by transitions**", trajectories will be sorted into two groups with different diffusive behaviour, based on the values of Pc. This sorting can be done (and re-done) separately afterwards. On the command window you can see the number of trajectory and the number of segments found. The results of the analysis of transitions is saved in a new folder: diff\ stab.

Menu **Calculate**:

Can be used to recalculate Pc or to redo the sorting if parameters are changed, i.e. the threshold to decide if the trajectory is stabilized (or confined) or not. Once the files .tnd were generated (Go button), the sorting of trajectories can be done separately.

Menu **Results**:

- **Summary of diffusion analysis:**

Compiles results of calculations of MSD (average values among all trajectories) and D for trajectories above the immobilization threshold, and Pc. Allows pooling between different data folders. If trajectories were localized, results are sorted into "in" and "out" trajectories and a dwell time is calculated (time spent in the domain).

- **Summary of stabilized events:**

Compiles results of Pc analysis, based on the sorting of trajectories done by "**sort by transitions**". It saves files containing Pc values, the calculation of the confinement area size L and the analysis of transitions between two states of diffusion behaviour ("stabilized" (~= confined) or "non stabilized"). It can also compile MSD and D results, sorting them as above. If trajectories were localized, there will be four categories in total: "in" and "out" domains, "stabilized" or not.

Please note that in this case all the trajectories are considered, independently of the immobilization threshold.

- **Plot trajectories and results**

"Plot trajectories" creates figure files that display the diffusion results for each individual trajectory. These Matlab figures can be saved as .tiff files if wanted. The packing coefficient vs time is saved as a .txt file for each trajectory.
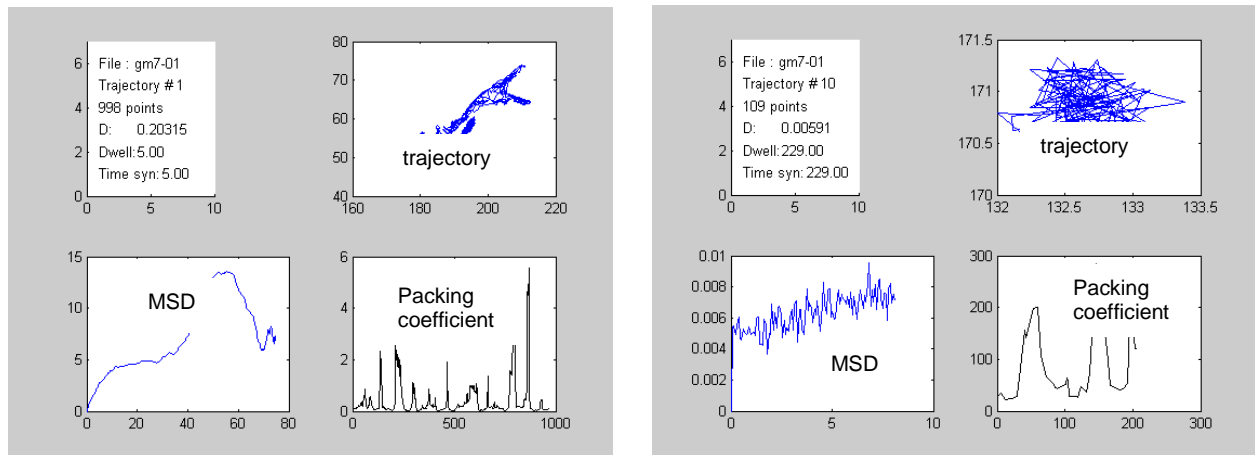
Format of diffusion analysis results:

<u>"diff" folder:</u>

Contains the results of diffusion analysis: **.tnd files** (one per movie) and a folder **/stab** if the analysis included sorting of trajectory segments by transitions of diffusive behaviour.

```
The structure of the .tnd file is:
```

`data(1).peri`	old definition of peri-domain area, not used anymore
`data(1).szpx`	pixels size
`data(1).till`	time between frames
`data(1).frames`	total number of frames
`data(1).nrotraj`	number of trajectories
`data(i).traj`	structure .traj:

`traj.coord`	trc data
`traj.msd`	MSD data
`traj.D`	D
`traj.b`	offset
`traj.fill`	packing coefficient
`traj.nrosegm`	number of segments
`traj.dwell`	total dwell time
`traj.trans`	starting point of the segment
`traj.segm`	structure with info about the segments:

`traj.segm(count).msd=`	MSD
`traj.segm(count).D`	D
`traj.segm(count).b`	offset
`traj.segm(count).data`	trc data

The summary files contain the compilation of:

- D (Dindomain.txt, Doutdomain.txt, Dinstab.txt, Doutstab.txt, Dinn.txt, Dinnostab, Doutnostab.txt):

1	1.0000000e+000	7.0000000e+000	1.0000000e+000	3.4767144e-002	1.2600000e+002
2	1.0000000e+000	7.0000000e+000	2.0000000e+000	1.0435604e-001	1.2600000e+002
3	1.0000000e+000	7.0000000e+000	3.0000000e+000	9.4687185e-002	1.2600000e+002
4	1.0000000e+000	7.0000000e+000	5.0000000e+000	5.3693080e-002	1.2600000e+002
5	1.0000000e+000	7.0000000e+000	6.0000000e+000	1.1035502e-001	1.2600000e+002
6	1.00				
7	1.00 **File #**	**traj#**	**segm #**	**D (µm²/s)**	**loc (number of domain in the mask)**
8	1.00				
9	1.0000000e+000	9.0000000e+000	7.0000000e+000	2.1042969e-002	1.0000000e+002
10	1.0000000e+000	9.0000000e+000	8.0000000e+000	3.7860378e-003	−1.0000000e+002
11	1.0000000e+000	9.0000000e+000	9.0000000e+000	2.5505248e-002	1.0000000e+002
12	1.0000000e+000	2.6000000e+001	1.0000000e+000	4.3873623e-002	4.4000000e+001
13	1.0000000e+000	2.9000000e+001	1.0000000e+000	3.2478226e-002	−1.8000000e+001
14	1.0000000e+000	3.2000000e+001	1.0000000e+000	1.9494648e-002	1.4000000e+001
15	1.0000000e+000	3.2000000e+001	2.0000000e+000	1.2271566e-002	−1.4000000e+001
16					

- cumulative distribution of D (Dcumout.txt, Dcumin.txt)

1	9.1671374e-09	0.0000000e+00
2	5.9576054e-05	2.5445293e-03
3		
4	**D (µm2/s)**	**cum frequency**
5	2.0609138e-04	1.0178117e-02
6	2.9990009e-04	1.2722646e-02
7	3.4376010e-04	1.5267176e-02
8	3.9185525e-04	1.7811705e-02
9	4.3618465e-04	2.0356234e-02
10	1.1031603e-03	2.2900763e-02

- average MSD (meanMSDin.txt, meanMSDout.txt, meanMSDinstab.txt, meanMSDoutstab.txt, meanMSDinnostab.txt, meanMSDoutnostab.txt)

1	0.0000000e+00	0.0000000e+00	0.0000000e+00	0.0000000e+00
2	7.5000000e-02	2.2196077e-02	1.0601568e-02	5.5491144e-04
3	1.5000000e-01	4.3759510e-02	2.3868388e-02	1.2493285e-03
4	**Time interval (ms)**	**average MSD (µm2)**	**SD**	**s.e.m**
5				
6	3.7500000e-01	9.3404871e-02	6.8992651e-02	3.6112404e-03
7	4.5000000e-01	1.0720142e-01	8.3901105e-02	4.3915846e-03
8	5.2500000e-01	1.2167120e-01	1.0289732e-01	5.3858919e-03
9	6.0000000e-01	1.3404879e-01	1.1915205e-01	6.2367034e-03

- Packing coefficient Pc (Pcout.txt, Pcin.txt):

1	1.0000000e+00	4.1683629e+03
2	2.0000000e+00	3.7112186e+03
3	3.0000000e+00	4.0113682e+03
4	4.0000000e+00	
5	5.0 **frame #**	**Pc (µm⁻²)**
6	6.0000000e+00	4.0430201e+03
7	7.0000000e+00	4.0369337e+03
8	8.0000000e+00	4.0921169e+03
9	9.0000000e+00	4.1236397e+03

- Confinement area size L (Lout.txt, Lin.txt):

1	2.3049408e+03	1.6531765e+00	4.4996269e+01	
2	1.2091261e+04	1.3220631e+00	2.0992447e+01	
3	**Pc**	**log L**	**L (nm)**	
4	4.1436652e+03	1.5560052e+00	3.4536044e+01	
5	1.2127548e+04	1.3214644e+00	2.0963530e+01	
6	2.8166059e+03	1.6131260e+00	4.1032316e+01	
7	4.5787629e+03	1.5160558e+00	3.2813749e+01	
8	3.8042974e+03	1.5530737e+00	3.5733351e+01	

L is calculated from Pc as described in Renner et al (2017).

- Duration and frequency of stabilization events (stabeventsextra.txt, stabevents.txt):

	movie	segments	events	# events	dwell time	duration events	frequency events	mean duration events
1	1.0000000e+00	5.0000000e+00	1.0000000e+00	1.0000000e+00	2.6100000e+02	1.5500000e+02	3.8314176e-03	1.5500000e+02
2	1.0000000e+00	6.0000000e+00	1.0000000e+00	1.0000000e+00	1.6300000e+02	1.6000000e+01	6.1349693e-03	1.6000000e+01
6	1.0000000e+00	4.1000000e+01	1.0000000e+00	1.0000000e+00	2.2600000e+02	7.9000000e+01	4.4247788e-03	7.9000000e+01
7	1.0000000e+00	7.8000000e+01	1.0000000e+00	1.0000000e+00	4.9000000e+01	1.3000000e+01	2.0408163e-02	1.3000000e+01
8	1.0000000e+00	8.8000000e+01	1.0000000e+00	1.0000000e+00	1.1110000e+02	7.7000000e+01	9.0090090e-03	7.7000000e+01
9	1.0000000e+00	1.0300000e+02	1.0000000e+00	1.0000000e+00	6.6000000e+01	2.6000000e+01	1.5151515e-02	2.6000000e+01
10	1.0000000e+00	1.1900000e+02	1.0000000e+00	1.0000000e+00	6.1000000e+01	2.1000000e+01	1.6393443e-02	2.1000000e+01
11	2.0000000e+00	1.0000000e+00	1.0000000e+00	1.0000000e+00	5.1000000e+01	2.0000000e+01	1.9607843e-02	2.0000000e+01

Frequency events: number events/frames. It can be used to calculate an effective kon.
Mean duration events: frames. It can be used to calculate an effective koff (Renner at al 2017).

- Percentage of stabilized trajectories (summaryeventsextra.txt, summaryevents.txt):

	# movie	% trajectoires stabilized	%temps stabilized
3	3.0000000e+00	2.6373626e+01	1.1461827e+01

-    Dwell time

|	# movie	Time inside (frames)	entries	Dwell time (s)
1	4.0000000e+00	7.0000000e+00	1.0000000e+00	5.2500000e-01
2	6.0000000e+00	8.6600000e+02	1.0000000e+00	6.4950000e+01
3	7.0000000e+00	2.3400000e+02	1.0000000e+00	1.7550000e+01
5	1.5000000e+01	2.9000000e+01	2.0000000e+00	1.0875000e+00
6	2.1000000e+01	7.3000000e+01	1.0000000e+00	5.4750000e+00
7	2.9000000e+01	1.7000000e+01	1.0000000e+00	1.2750000e+00
8	3.4000000e+01	3.4000000e+01	1.0000000e+00	2.5500000e+00
9	4.4000000e+01	2.2000000e+01	1.0000000e+00	1.6500000e+00
10	5.4000000e+01	2.4000000e+01	1.0000000e+00	1.8000000e+00
11	6.3000000e+01	4.6000000e+01	1.0000000e+00	3.4500000e+00

<u>"Dinst" folder:</u>

It is created by the analysis "D vs time" in the Diffusion menu on SPTrack_v6 window. As a result of this analysis, there will be '-dinst.txt' files with:

gav02sdinst

	Trajectory[X]	Frame[Y]	x[Y]	y[Y]	loc[Y]	loc2[Y]	Dinst[Y]	angle[Y]
1	1	1	127,45871	91,64334	0	0	0,00318	-1,79139
2	1	2	127,41445	91,63341	0	0	0,00208	-2,51609
3	1	3	127,30888	91,48724	0	0	4,83111E-4	-2,86541
4	1	4	127,30161	91,46159	0	0	6,74213E-4	2,70295
5	1	5	127,36097	91,33506	0	0	0,001	-0,75032
6	1	7	127,29957	91,40093	0	0	0,00121	2,38631
7	1	8	127,3243	91,37466	0	0	0,00125	-0,05309
8	1	9	127,31722	91,50789	0	0	4,34827E-4	0,05162
9	1	10	127,31814	91,5257	0	0	6,30133E-4	0,2499
10	1	11	127,34049	91,61326	0	0	0,00121	3,02985
11	1	12	127,35322	91,49981	0	0	0,00151	-1,29082
12	1	13	127,33061	91,50632	0	0	8,88579E-4	0,30334
13	1	14	127,385	91,68008	0	0	8,88622E-4	2,04864
14	1	15	127,48709	91,62722	0	0	0,00132	-1,25999
15	1	16	127,35435	91,66985	0	0	0,00133	1,45022

X and Y are the positions of the trajectory, loc and loc2 have the information about localization (optional), Dinst is the D in time and the angle is the direction of the next displacement.

Basic stats

The menu option "Statistics" opens a new window that calculates some basic and common statistics for diffusion analysis:
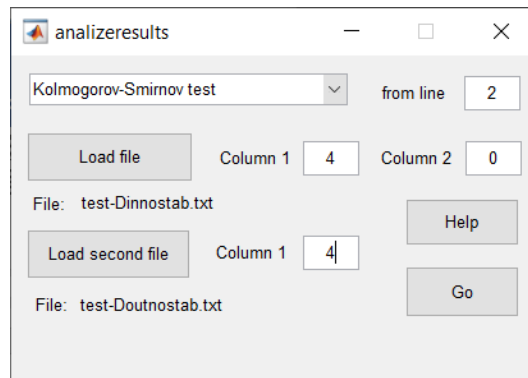
You can open .xls or .txt files (**Load file**, **Load second file** if needed). You have to indicate the starting point of data (**from line**) and the columns to be used (**Column 1**, and eventually **Column 2**). To de-select a file, click on '*annuler*' ('cancel') of the window that appears after clicking on Load...

The possibilities of the rolling list are:

- **Cumulative distribution**
  Makes a cumulative distribution on one data column of one file, assigning a value from zero to one at each data value and saves in a separate .txt file.

- **Kolmogorov-Smirnov test**
  KS test between two column of the same file, or two columns from different files.

- **Student t-test**
  t-test between two column of the same file, or two columns from different files.