**NOBIAS Quick Start Guide** 

Thanks for your interest with using NOBIAS!

The NOBIAS algorithm (NOnparametric Bayesian Inference for Anomalous diffusion in Singlemolecule tracking) is a two-module algorithm for analysis of multi diffusive state SPT dataset

and predict the anomalous diffusion type for each state.

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NOBIAS is written and test on MATLAB R2018b.

NOBIAS requires the lightspeed toolbox. (https://github.com/tminka/lightspeed)

Please put the lightspeed to you MATLAB path, then change current folder to the directory of lightspeed and run: install lightspeed command.

The running of lightspeed need C/C++ compiler for Windows, if not yet installed, please try to use 'MATLAB Support for MinGW-w64 C/C++ Compiler', open the MATLAB add-ons and search for 'MinGW' to get this compiler, or see the Github page of lightspeed for more options.

There are two module of NOBIAS, the first HDP-HMM module and the second RNN module. The second module depends on a pre-trained classification network and the output from the first module.

To use the HDP-HMM module, use command:

```
out = NOBIAS(data);
```

Please load 'example\_data.mat' in the main NOBIAS folder and run the above command to test that NOBIAS is installed correctly.

The input data variable should be a structure variable with at least two fields: 'obs' and 'TrID', 'obs' would be a 2 by T matrix where T is total amount of steps and 2 indicates 2D tracks. Elements should be the step displacement. 'TrID' should denote which tracks the step is from

and should be a 1 by T integer variable. To do the motion blur corrections, input 'data' should also have a field 'cor' obs' that denotes the correlation steps.

To prepare your experimental tracks to the input format, please use command:

```
data = NOBIAS preparedata(AllTracks);
```

Input 'Alltracks' is a N by 1 cell array, where each element of the cell array is a tracectories with at least 4 colmns, where the 2nd columns should correspond to the frame of the tracks (gaps are allowed and should be pre-filtered in tracking step to avoid too huge gaps), the 3rd and 4th columns should be the rows and columns of the 2D tracks coordinates, pay attention to x-y and row-col difference. Change 'Params' field 'frametime' and 'pixelsize' according to your experimental settings.

For standard simulated tracks, NOBIAS uses  $\alpha = 1$ ,  $\gamma = 0.1$ ,  $\kappa = 5$ , for tracks with motion blurring,  $\kappa$  should be increased accordingly if you observed final results have states number that share very similar D. For example, for a averging-10-step motion blurring tracks  $\kappa = 20$  is used for a final 10-step motion blur tracks, and  $\kappa = 100$  is used for 100-step motion blur tracks.

 $a = 1, \gamma = 0.1$  can also be changed and see (Fox et al., 2008) for details about what they means.

To run simulated tracks, use the two inbuilt simulation codes NOBIAS\_step\_simu\_standard and NOBIAS\_step\_simu\_blur to simulate a standard BM mixture or Motion Blurr mixture.

To run the RNN module, use the following commands:

```
load('net.mat')
state_model=predict_state_model(out,data,net, minseglength);
Model_Prob = NOBIAS_difmodel_plot(state_ model, out)
```

A net need to be per-trained for RNN module. There are two nets in NOBIAS/RNN\_part trained with 20 steps and 40 steps trajectories.

Model\_Prob rows stand for each diffusive state and columns stands for prediction probability of the diffusion type BM, FBM, CTRW, LW.

If you don't have any track segments that is at least 'minseglength' long for one of the diffusive state, the RNN module could give error/NA value.