



**1:** In the case of synthetic data, the user can generate time traces by clicking on the “Generate” key. Pressing this key automatically disables the rest of the app and a new pop-up window will ask about the parameters that the user wants to use. These parameters will be shown in parameter lists 7 and 8. The generated time trace will be shown in plot 11. Also, the ground truth of the trajectories will be shown in plots 12, 13, and 14.

\*Note: Parameters of the priors are fixed! These parameters are set in a way to make sure the effect of the prior is negligible compared to the likelihood.

**2:** In the case of importing a trace, the user can press this key and a new window will ask about the parameters and the file. The imported file can be in ‘.mat’ or ‘.txt’ file. The order of the data should be a matrix with 3 rows (micro-times (ns), macro-times (s), channel index (1 or 2) ). The imported parameters will appear in parameter list 8. The imported time trace will be shown in plot 11.

\*Note: Importing traces requires known parameters of IRF for both donor and acceptor channels, and the pulse period.

**3:** After generating or importing the time trace, the “Run” key will be available. Pressing this key leads to a pop-up window asking for three parameters listed in lists 9 and 10. All keys will disable while the program is running and gauge 18 will show the progress of the algorithm. After finishing the analysis, the results will show in plots 12, 13, 14, 15, 16 and 17. Also, the “continue” will be available for extra analysis.

**4:** The “Continue” key will be available after the “Run” key. This key will allow the user to run the algorithm for larger iterations. After pressing this key, a pop-up window will ask the user about the number of extra iterations. While the algorithm is running the progress of the algorithm will be shown in gauge 18. After finishing the analysis, the results will show in plots 12, 13, 14, 15, 16 and 17. Also, the “continue” will be available for extra analysis.

**5:** The “Help” key will open the help pdf (this document).

**6:** The “License” key will open the license document.

**11,12,13,14,15,16, 17:** These are the plots related to the illustration of the time trace and the results of the algorithm.

**7, 8, 9, 10:** These are the list of the parameters that the data is generated or input by the user.

**18:** This gauge shows the progress of the algorithm.

**Extra:** Final results will be saved in a file ‘param.mat’ in the directory. This file contains all the output of our analysis and can be used in the source code to be visualized.