The two enclosed .m files (mybttnfcn\_.m and Seg2Analysis4Fluor0.m) were developed based on Matlab R2017a. These two files should be placed in the matlab folder. In the mybttnfcn\_.m file, line 22 should be replaced with the direction where these two files are placed. This will be called when Seg2Analysis4Fluor0.m is running. In the Seg2Analysis4Fluor0.m file, line 98 and 102 should be replaced with a direction where people want to save the analyzed files in their computer.

To use these codes, open the Seg2Analysis4Fluor0.m file in matlab.

1) Place the typical trace in the same direction and click "run" button.

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描述已自动生成

2) A dialog box will be open and please double click the typical trace file.

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3) Then another dialog box and a figure panel will appear. **Do not close any of them** and click the figure panel to place it front.

图形用户界面

描述已自动生成

4) In the figure panel, a green trajectory of Cy3 fluorescence and a purple trajectory of SNAP649 fluorescence are presented. Use the zoom in or out tool to zoom the region where Cy3 disappears and SNAP649 appears, which represents transcription termination upon Sen1 action.

图形用户界面, 应用程序

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5) Click the left button once at the time point where SNAP649 appears, click the left button again at the time point where Cy3 disappears, and click the left button at the time point where SNAP649 disappears. These actions select three points which give two segments corresponding the time intervals before and after transcription termination, and these three point will be saved in the matlab code.

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6) Click the right button finally to tell the code that your analysis on selecting points on this trajectory is finished. Two segments will be lined between those points.

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7) In the dialog box, a "Points.txt" file is created containing the three points you selected. Double click this file, and the code will continue to run to calculate the two time intervals and their average FRET values. The figure panel will then be saved and closed automatically.

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8) In the direction in your computer, a .fig and a .txt file named with the name of the analyzed .txt file followed with "\_SegAnalysis4Fluor" will be created. The .fig file can be opened with Matlab saving the point selections in the analysis. The .txt file contains the analyzed results, in which the 1st column is the file name, the 2nd-4th columns are the time values of the original three points, the 5th-7th columns are the nearest time values of the three points on the trajectory, the 8th and 9th columns are the two time intervals, and the 10th and 11th columns are the mean FRET values of the two time intervals. We are interested in the lifetimes of the coexistence of Cy3 and SNAP649 fluorescence.

