

# FRET Automation

## Trace Picking

There are two programs to automate the trace picking process – **FRET\_Picking** and **FRET\_Picking\_Scriptable**. Both work identically, the only difference being how the function arguments are input. Specifically the signal output channels/ dye pairs used. The other three parameters, M, N and CUT, are adjustable parameters in the picking process and are detailed below.

These programs should be executed in the directory containing your “Run” files. For each run file, it will pick traces based on the relevant parameters and save the picked traces to a newly created directory labeled “PickedTraces\_ChI\_ChJ\_M\_N\_CUT”, where I and J are the channels selected. Picked traces will be saved into a new MATLAB session file with a “-p” ending.

If this is your first time analyzing data for a particular type of experiment, it might be worth it to try out several parameter values to determine ideal values. Write a script calling the **FRET\_Picking\_Scriptable** function with a variety of inputs and allow it to run overnight.

### **FRET\_Picking(M, N, CUT)**

M – Size of initial smoothing window in frames.

5 appears to be a good number. Less for shorter frame rates or faster transitions.

N – Size of transition measure window.

20 appears to be a good number. Smaller for faster transitions, larger for noisy data

CUT – The cutoff value that will be used to determine whether or not a FRET transition has occurred.

200 appears to be a good number. Less if not many transitions have been found or if your traces have low intensity transitions. More if you are getting a lot of junk/ noisy traces.

Once the program is run, the user will be prompted to input channel information.

### **FRET\_Picking\_Scriptable(I, J, M, N, CUT)**

I – First signal channel to be analyzed

1 for Cy3, 2 for Cy3.5

J – Second signal channel to be analyzed

3 for Cy5, 4 for Cy5.5

M – Same as above.

N – Same as above.

CUT – Same as above.

## State Assignment

The state assignment process has been semi-automated through the **FRET\_AssignStates** program. The program will attempt to determine FRET transition points and assign state values between transition points. However, the state assignment process is nowhere near perfect and needs a good bit of human correction. The program has been modified from its original format to make state assignment/reassignment more convenient.

There are three new arguments to the program – M, N and CUT. They are identical to the parameters used in **FRET\_Picking** and **FRET\_Picking\_Scriptable**, however I have found that the state assignment process is more sensitive, and different (lower) default parameter values seem to be more appropriate (detailed below).

The program is written, as it was previously, so you can quit out mid-way through and your work is saved in a temp file. Re-running that temporary file allows the user to begin where they left off. Additionally, this allows users to adjust transition picking parameters – M, N, and CUT – in the middle of an assignment session without ruining previously assigned states.

Note that, when the program is run, it first performs a calculation on each trace in an effort to find FRET transitions. How long this calculation process takes will depend on the number of picked traces. I have estimated that it takes as long as 1 minute for every 600 picked traces.

### **FRET\_AssignStates(settings, M, N, CUT, BLEACH\_CUT)**

settings – settings\_now object.

This is the same settings\_now object utilized in the previous version of the state assignment program, that contains the file name information as well as a few flags. Note that the hmmFlag should always be set to 0.

M – Size of initial smoothing window in frames.

5 appears to be a good number. Less for shorter frame rates or faster transitions.

N – Size of transition measure window.

15 appears to be a good number. Smaller for faster transitions, larger for noisy data. Slightly smaller than in the **FRET\_Picking** programs in an effort to catch quick high FRET to low FRET transitions.

CUT – The cutoff value that will be used to determine whether or not a FRET transition has occurred.

40 appears to be a good number. Less if not many transitions have been found. More if you are getting a lot of junk. Note that this is much lower than was used in the **FRET\_Picking** programs. That is because you want the program to recognize high FRET/low FRET transitions, which are less intense. Additionally, at this step, it is slightly better to

have it pick up more false transitions (within reason) rather than miss actual transitions.

BLEACH\_CUT – The cutoff value that will be used to determine whether or not a bleaching event has occurred.

100 appears to be a good number. Less if bleaching events are being missed, more if it is placing them too liberally.

## User Interaction Commands

This point in the program will require some user interaction. For each trace, transition points have been identified, either by the program or by user entry, and states are defined between those transition points. State assignments can be made, and transition points added or removed, using the following commands. Note that transition points are plotted as vertical blue lines along the x-axis of the bottom plot. Some traces may not have any predicted state assignments but will still have predicted transition points.

### **FRET\_AssignStates** Commands:

#### New Commands:

##### Change State

Double mouse click, followed by an integer – This is how state assignments are made in the new program. Double clicking indicates between which transition points you want to redefine the state, and the integer entered is the new state definition.

##### Insert Transition

“t”, followed by a double mouse click – Will insert a new transition point at location of the mouse click. Note that no new state assignment will have been made, this will have to be done separately.

##### Reset Transitions/ States

“r”, “R” – Will erase all previously defined transitions (Reset).

##### Original Commands

“o” – This will take you back to the Original state assignment procedure. Double click to lay down the initial transition point, then a second click to lay down the second transition point, then an integer to define the state in-between. It will also erase all previously defined transition points between the newly defined points. This is meant to be a way to erase a series of poorly marked transitions, without having to start over from scratch.

#### Old Commands: (These have not changed)

“c” followed by an integer – change color

1 is the green dye channel (for bleaching events)  
3 is the FRET channel  
2 and 4 are currently unused  
“<” (really “.”) – back to previous trace.  
“>” (really “;”) – forward to next trace.  
“x” followed by mouse click – zoom x-axis.  
“y” followed by mouse click – zoom y-axis.  
“q” – quit mid-assignment and create temp file.