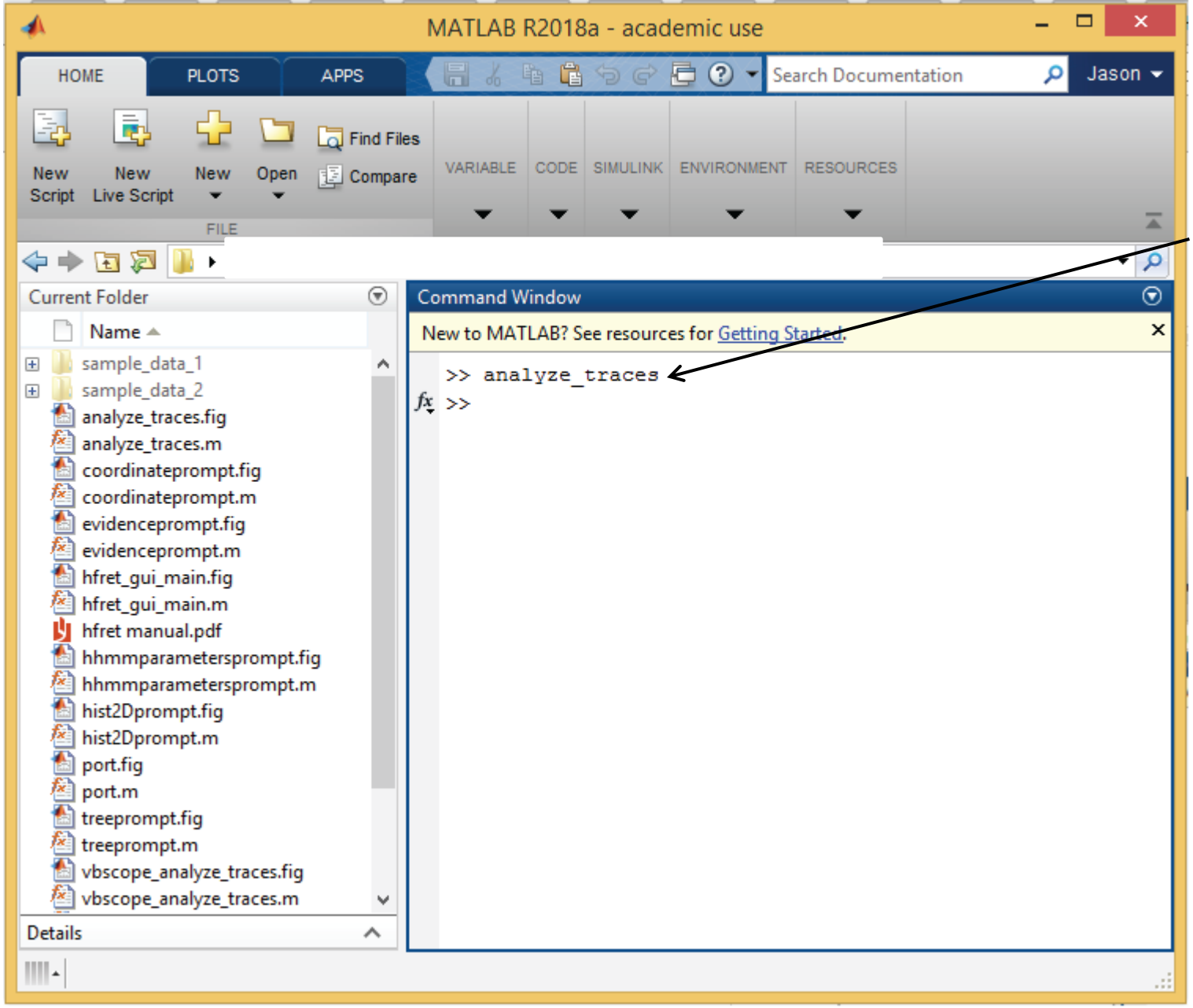
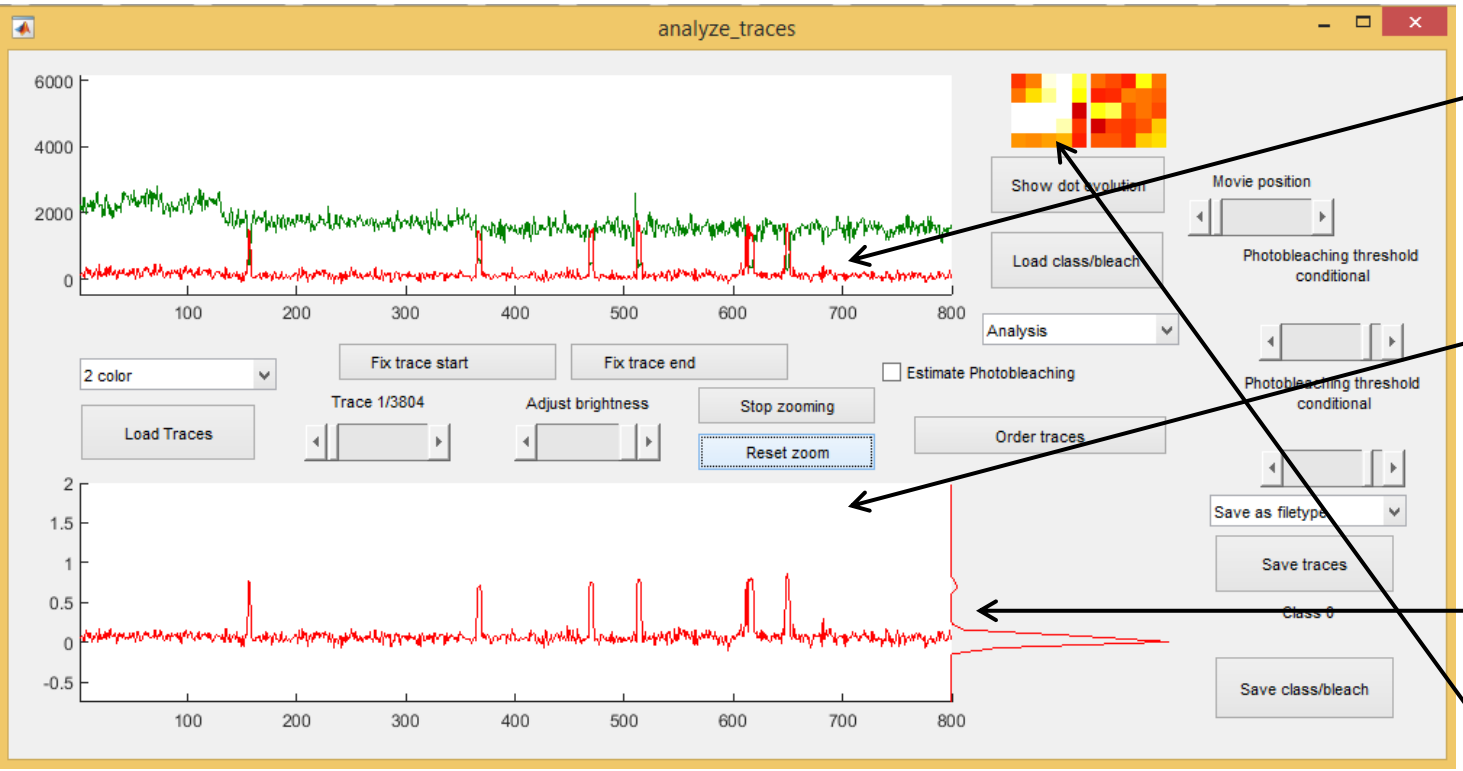
hFRET manual



Open the trace viewer gui by typing as shown and pressing <Enter>

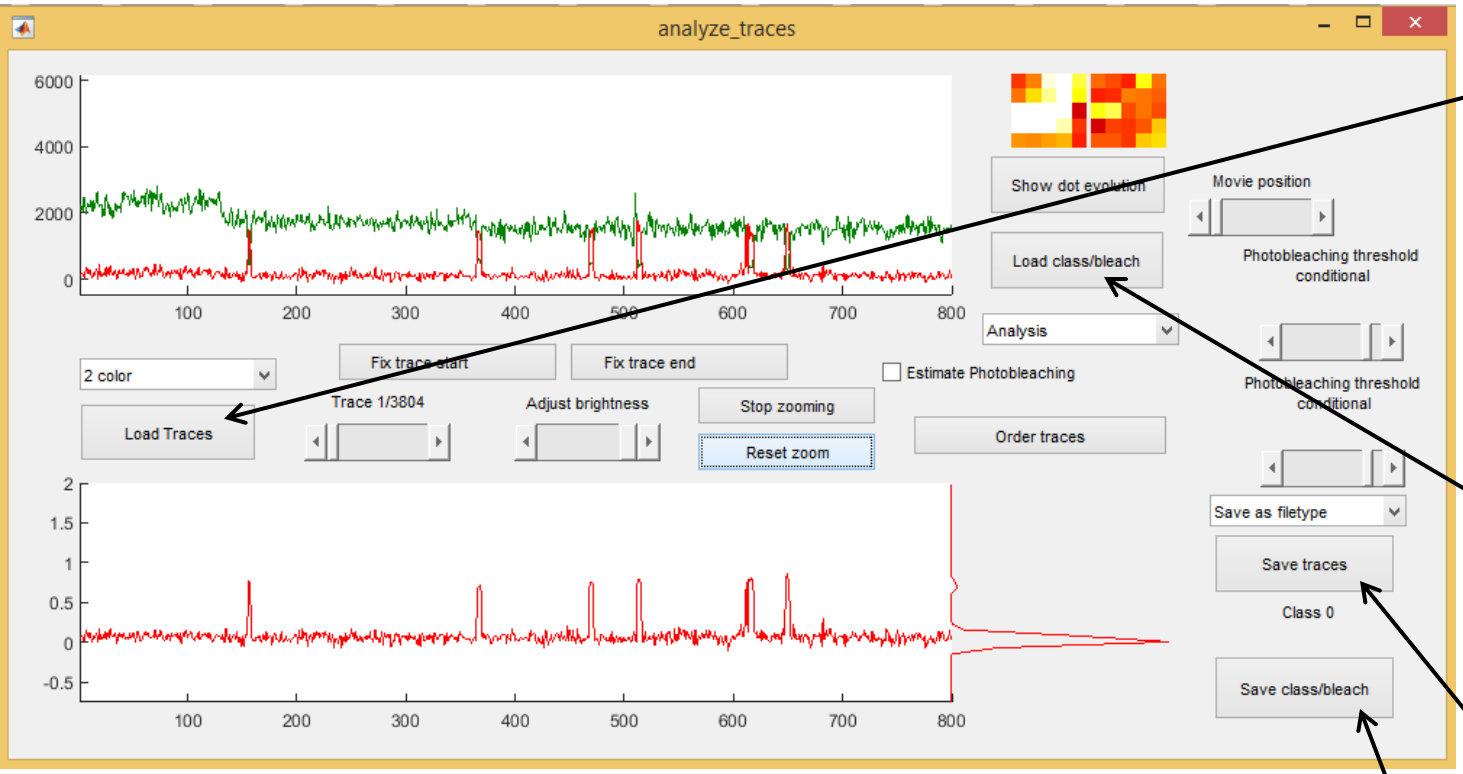


Intensities of each color

Fractional Intensities (for two colors, potentially EFRET)

Histogram

Chromophore context

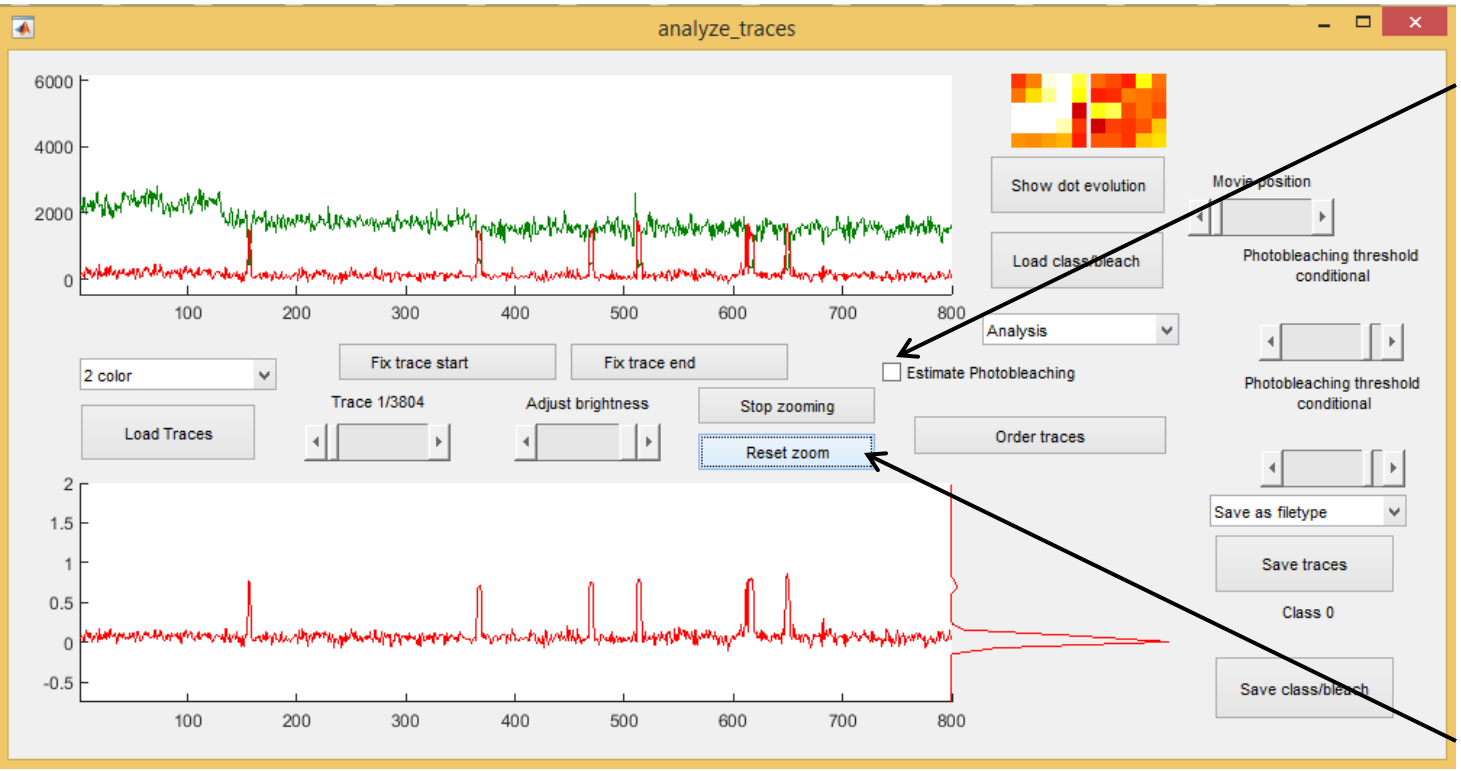


Load in data (can take multiple files; number of colors must be specified above first)

Load in classifications (can take multiple files)

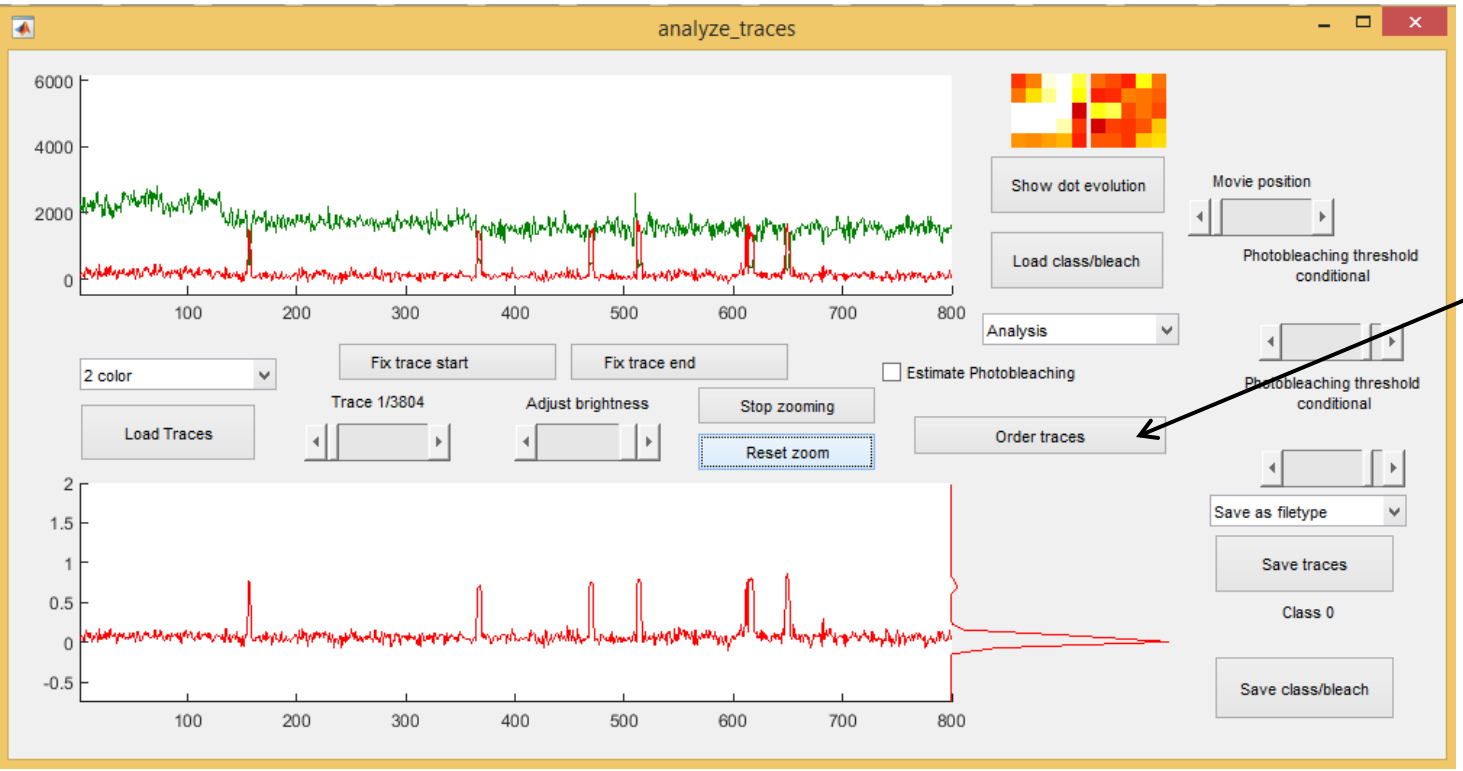
Save trajectories (.dat, just traces; vbscope – everything.)

Save classifications



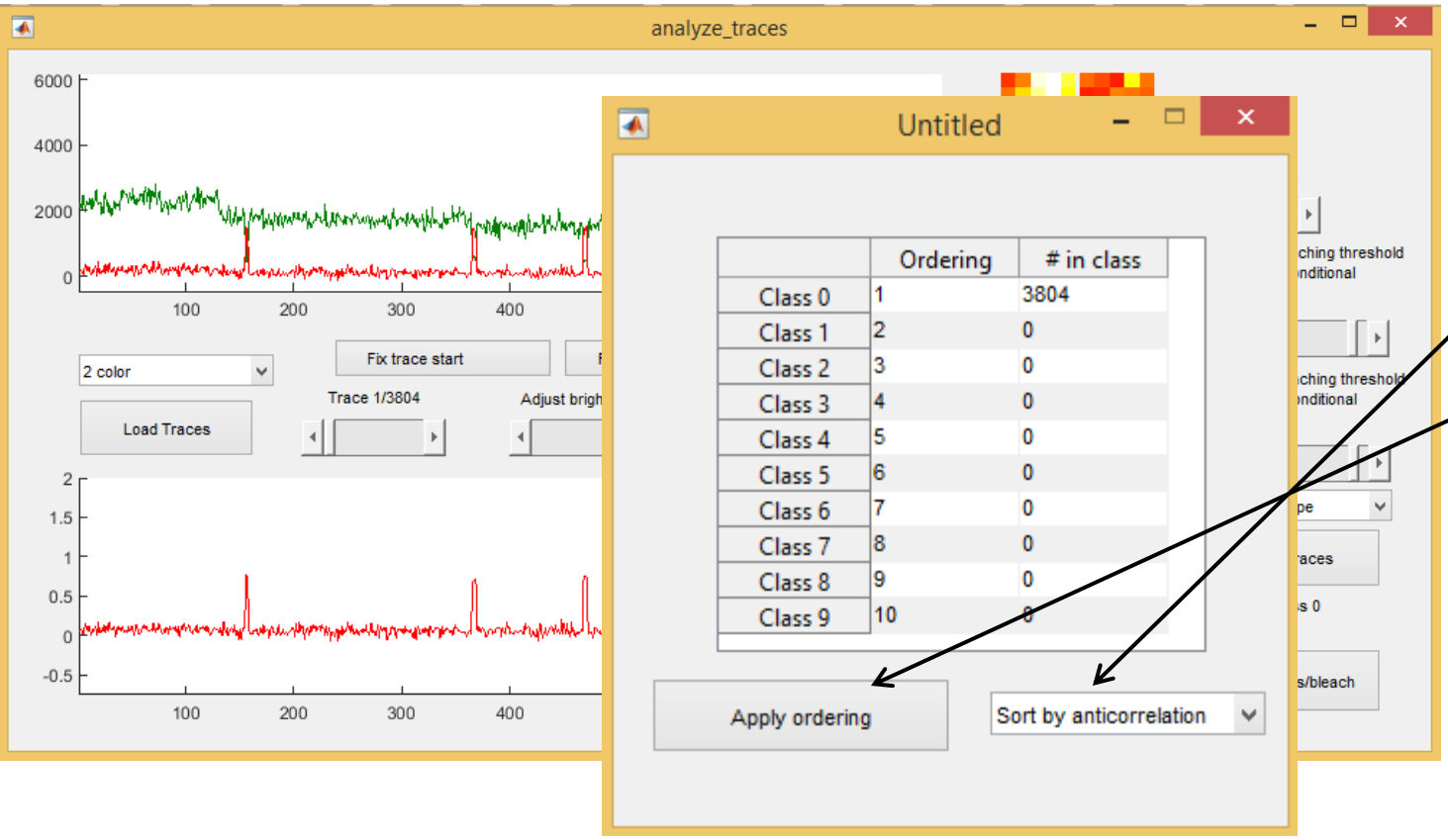
Enable photobleach detection/correction

Keyboard shortcuts:
s: fix start (then shift-click each color)
f: fix finish
n: next trace
b: back one trace
z: zoom in. When done must hit “stop zooming”, here.



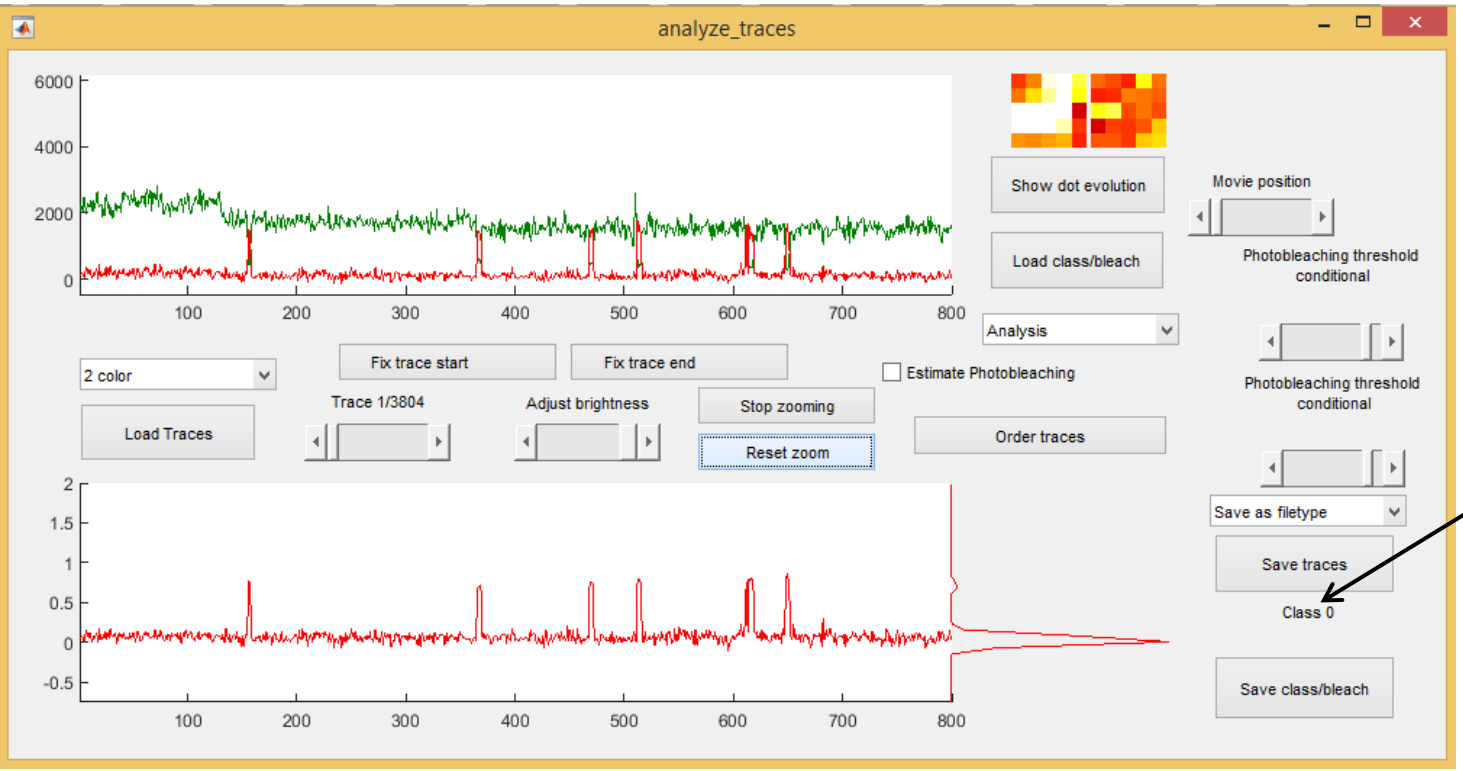
Sorting through the traces

Automatic sorting



Sorting through the traces

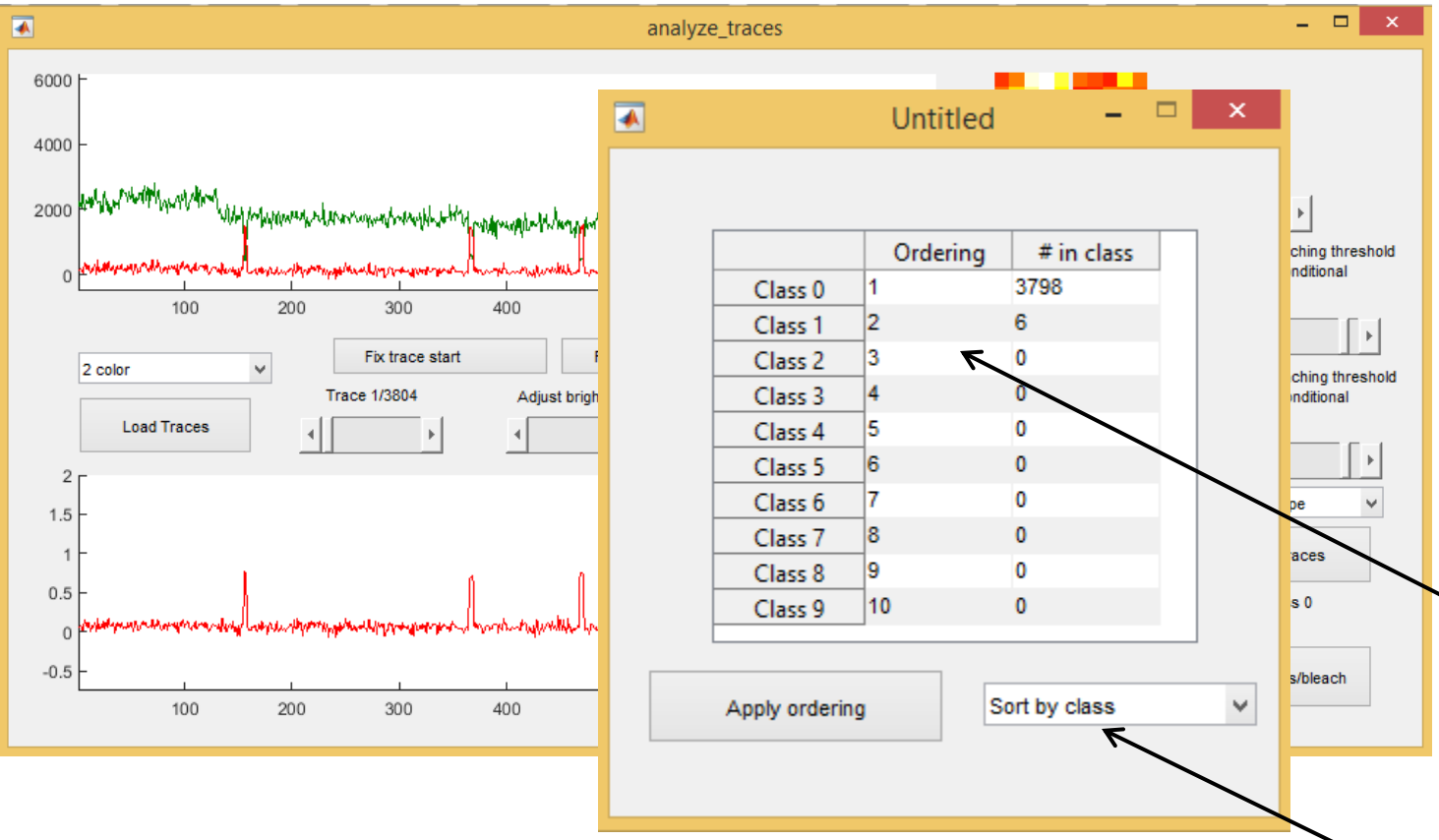
Calculates anticorrelation. Clicking “Apply ordering” places the traces in order form the most to the least anticorrelated.



Sorting through the traces

Manual sorting

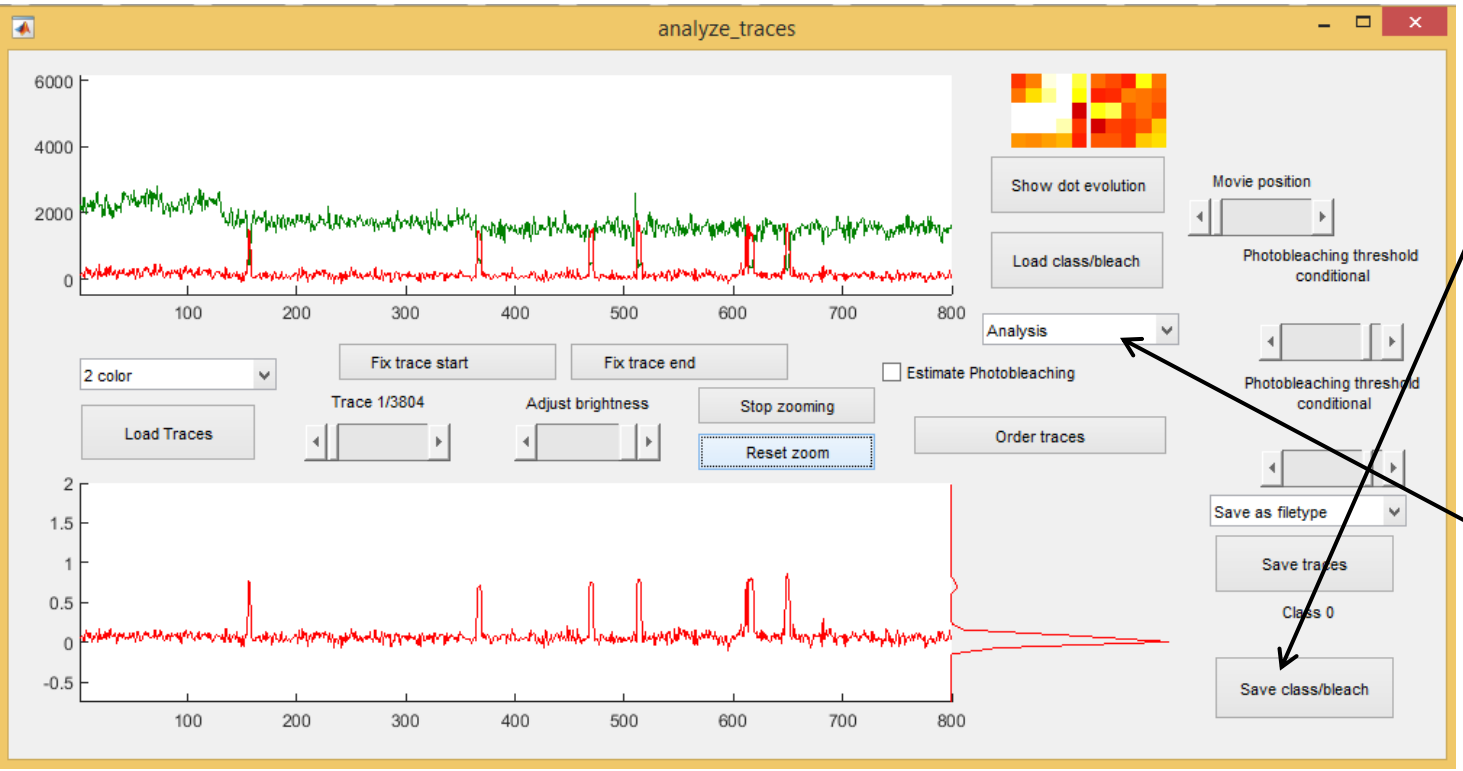
Click a number between 0-9 after clicking a graph. The trace is now in that class. Readout is here.



Sorting through the traces

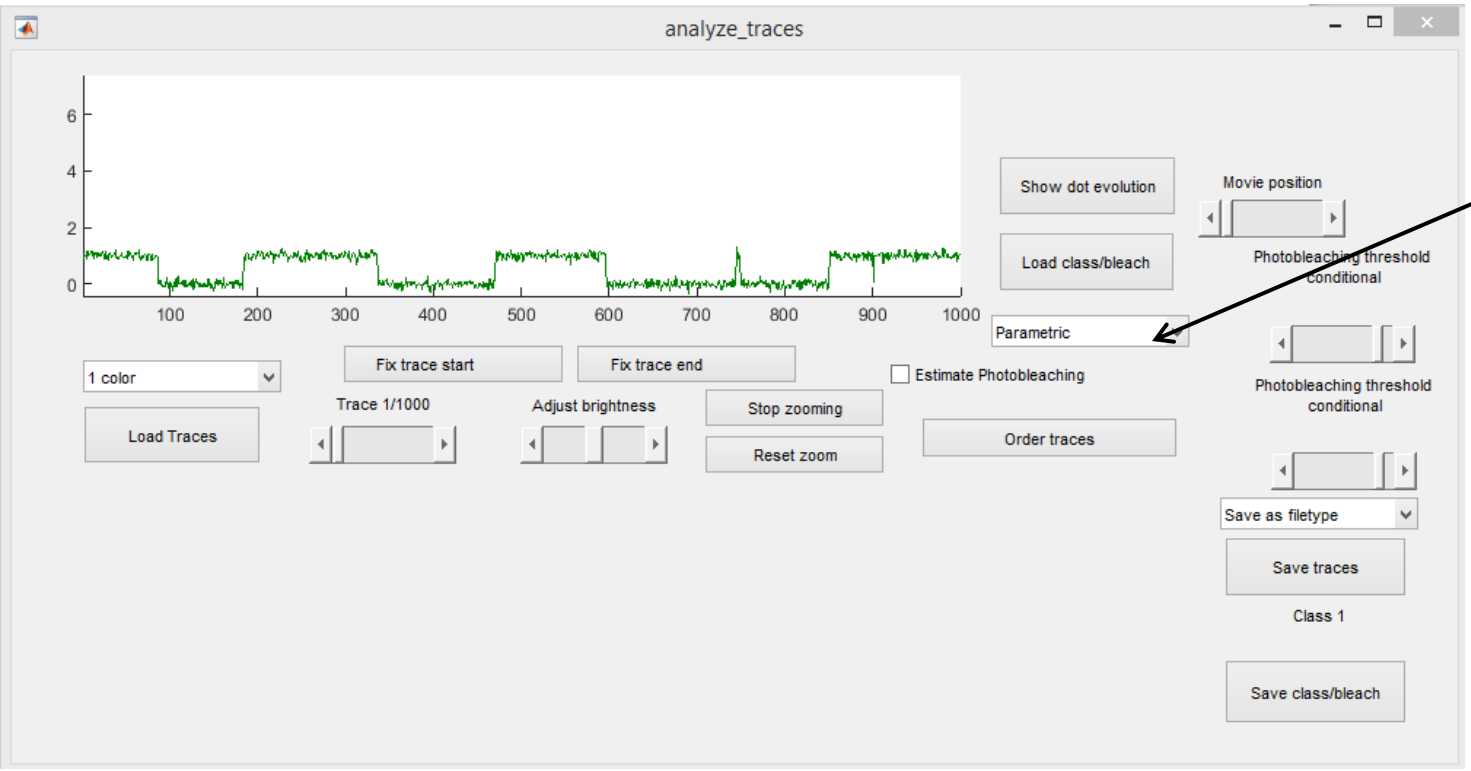
Manual sorting

Clicking “Ordering traces” again, this table appears. The “Ordering” column is editable and allows the classes to be put in order from 0-9 (1-10), if “Sort by class” is selected when ordering is applied.

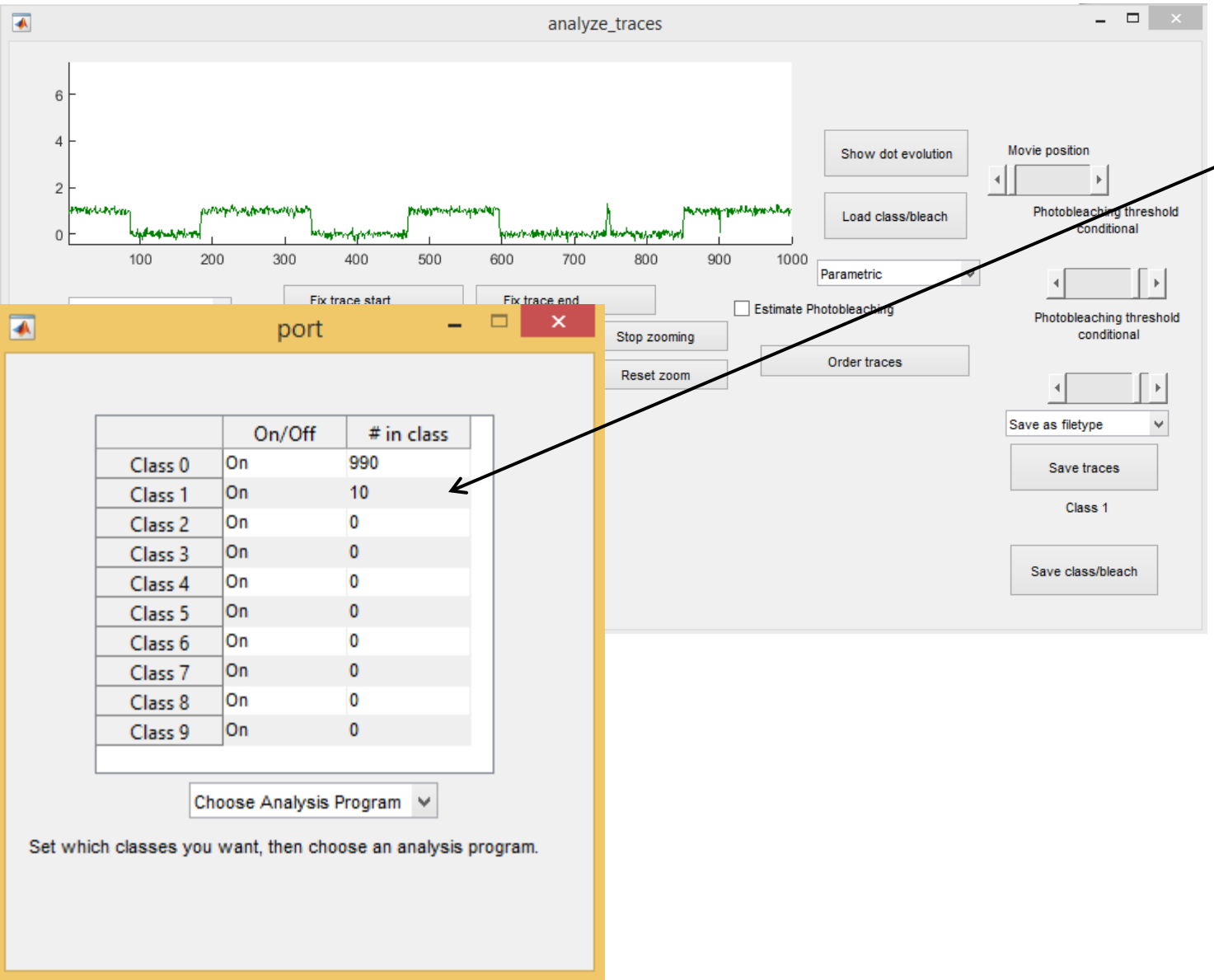


Once sorting/start/stop correction is complete, save class/bleach

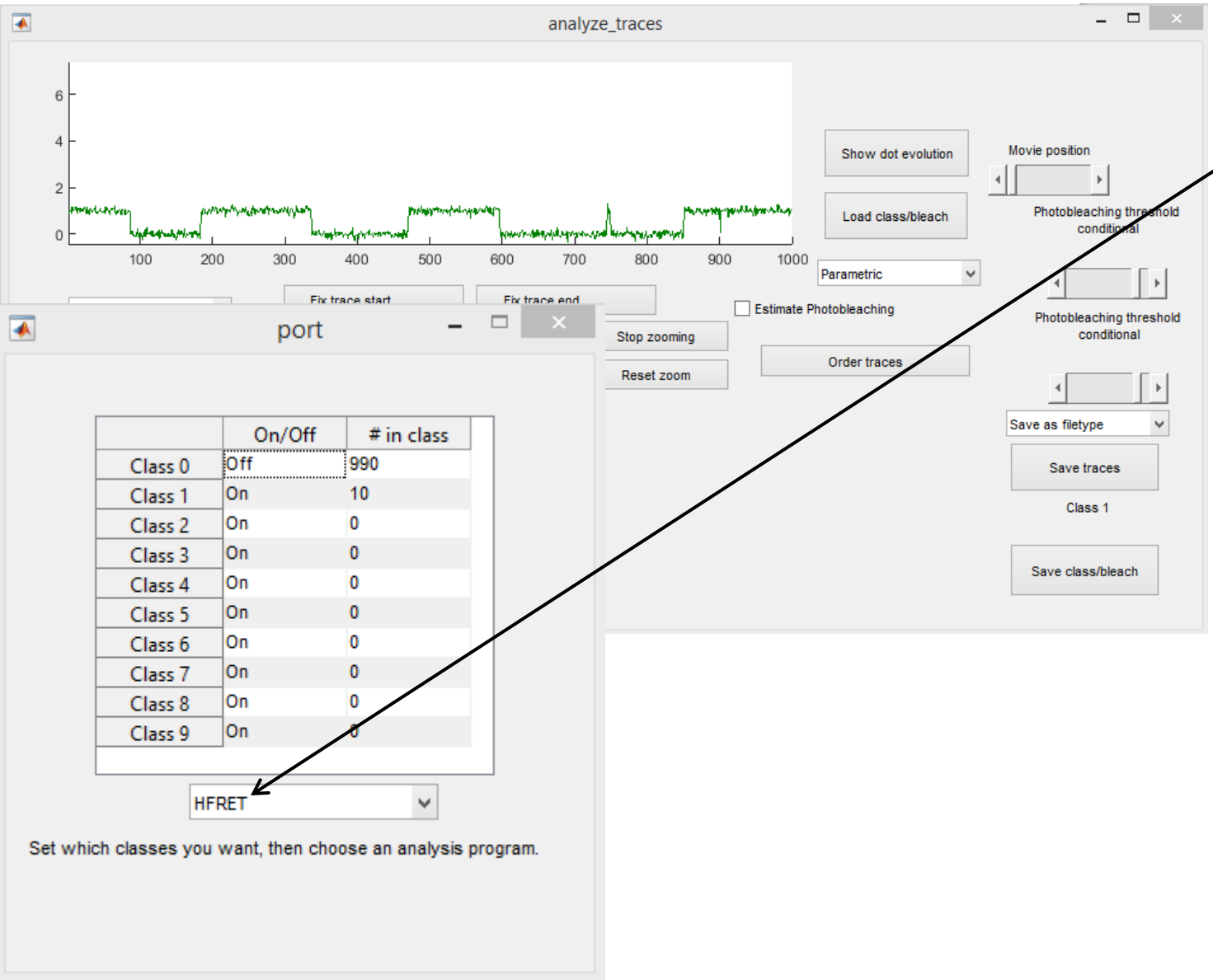
Next open a parametric or a nonparametric analysis type



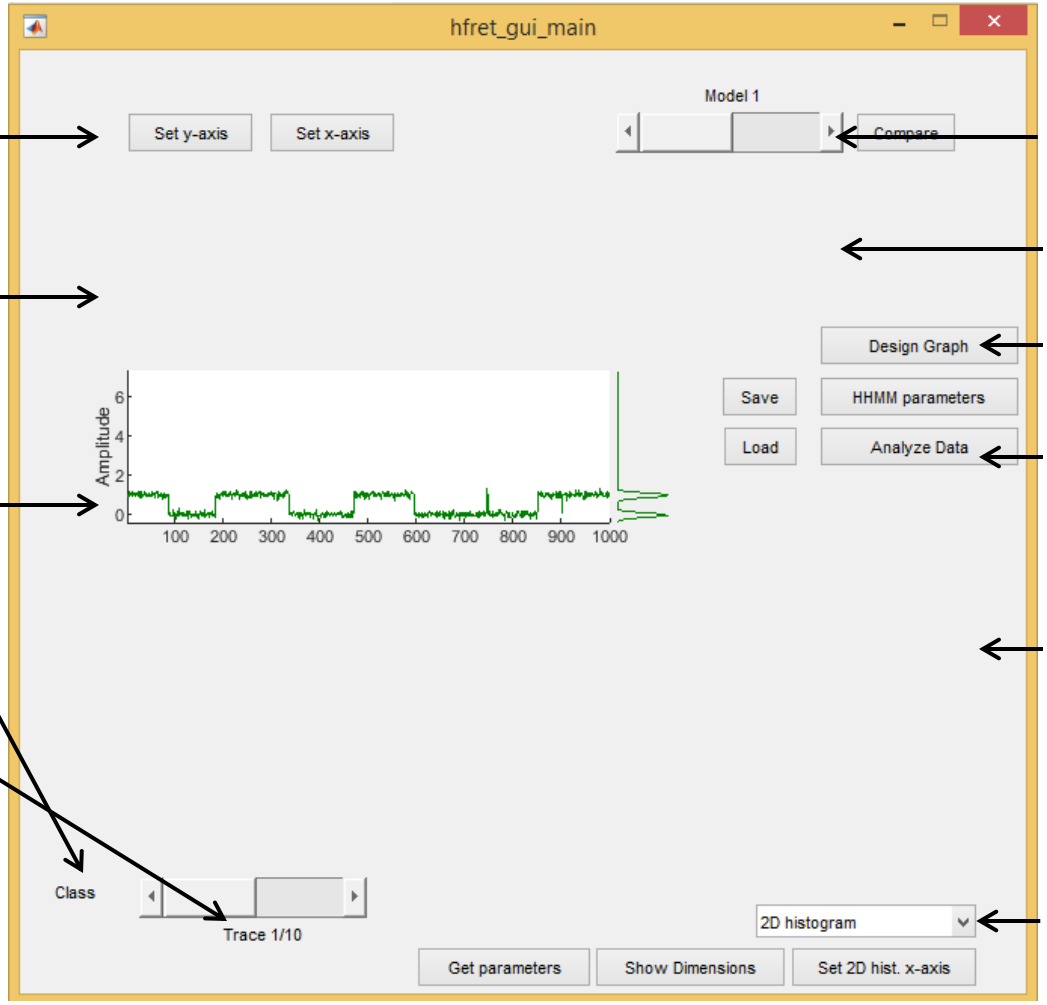
Change from “Analysis” to “Parametric”



Choose which classes to port over by clicking on the table and toggling a class “on” or “off” by hitting the appropriate number on the keyboard



For hFRET, choose "HFRET"



Axis adjustments

Trajectory (below, if only one dimensional

Trajectory (fractional units)

Class

Plotted trajectory

Current model

Model topology

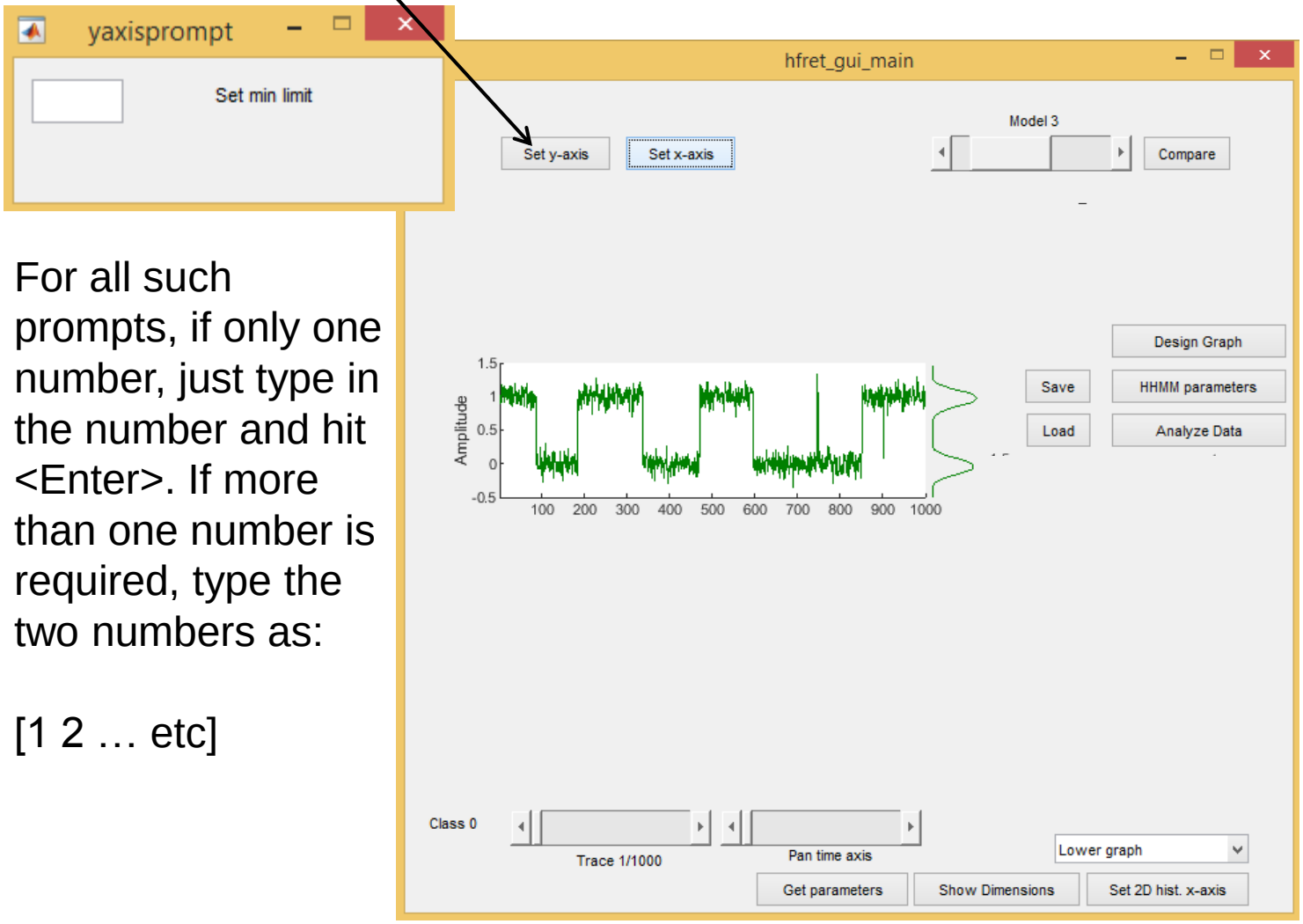
Model parameters

Begin analysis

Population plots

Population plot parameters

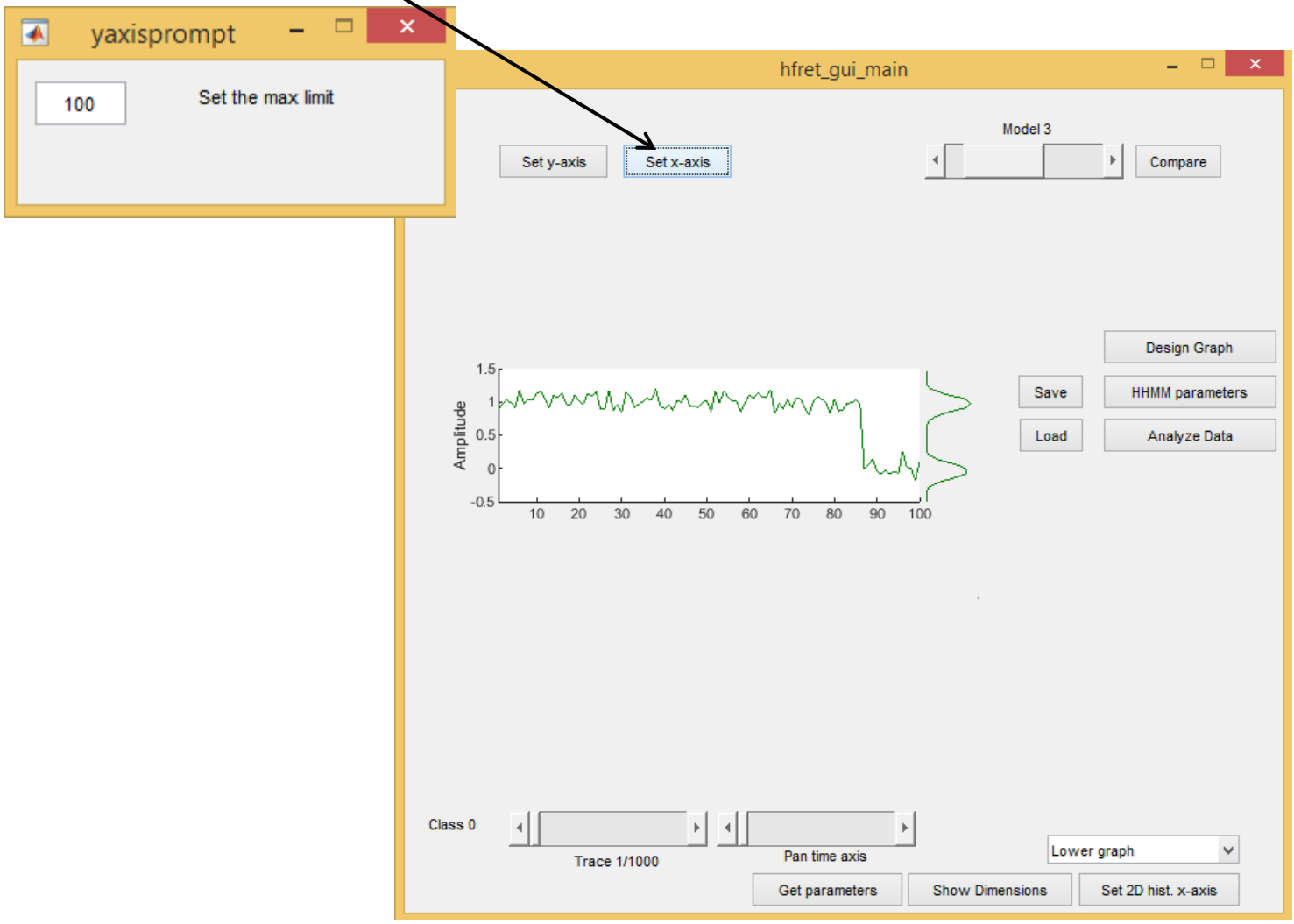
Y axis adjustments

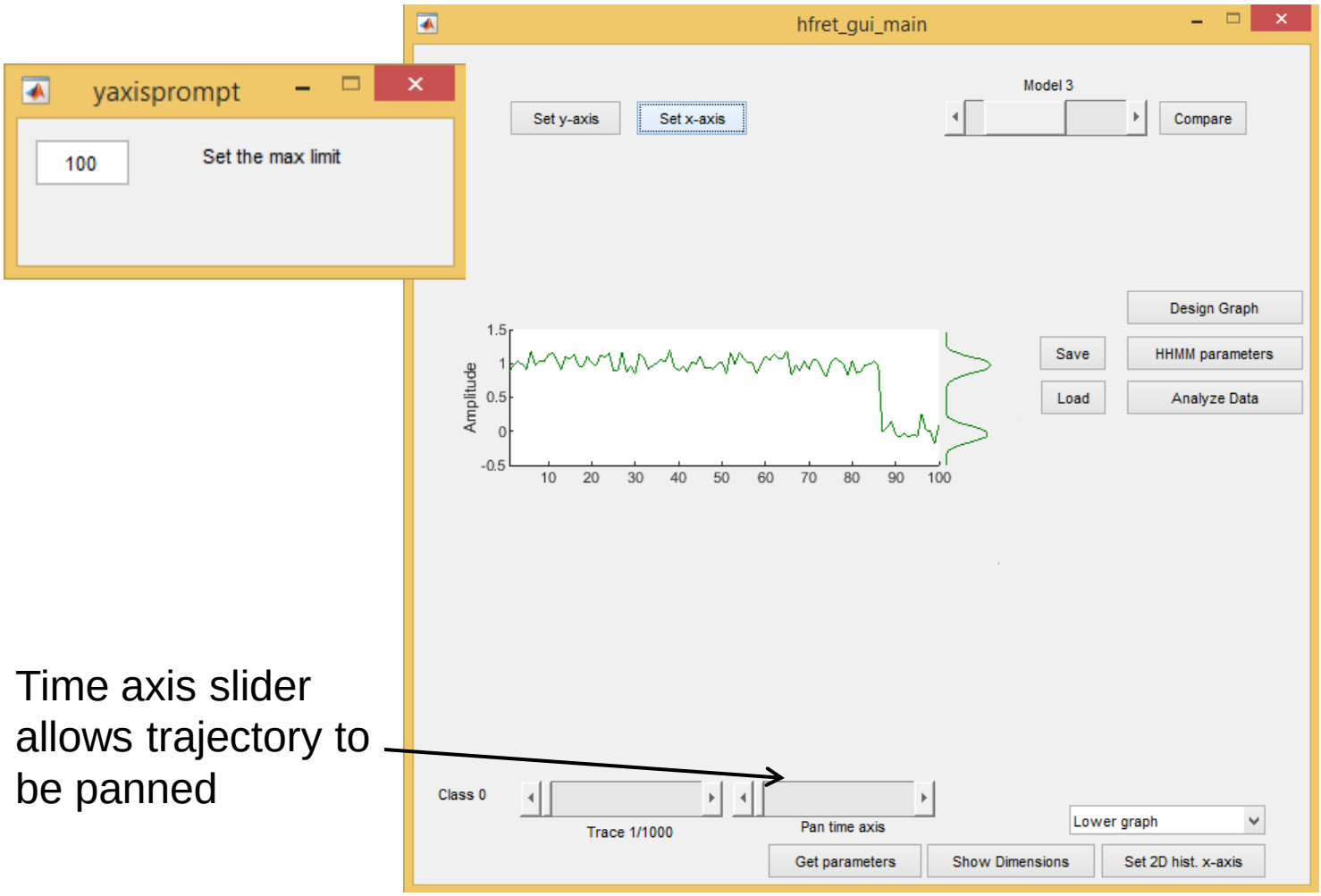


For all such prompts, if only one number, just type in the number and hit <Enter>. If more than one number is required, type the two numbers as:

[1 2 … etc]

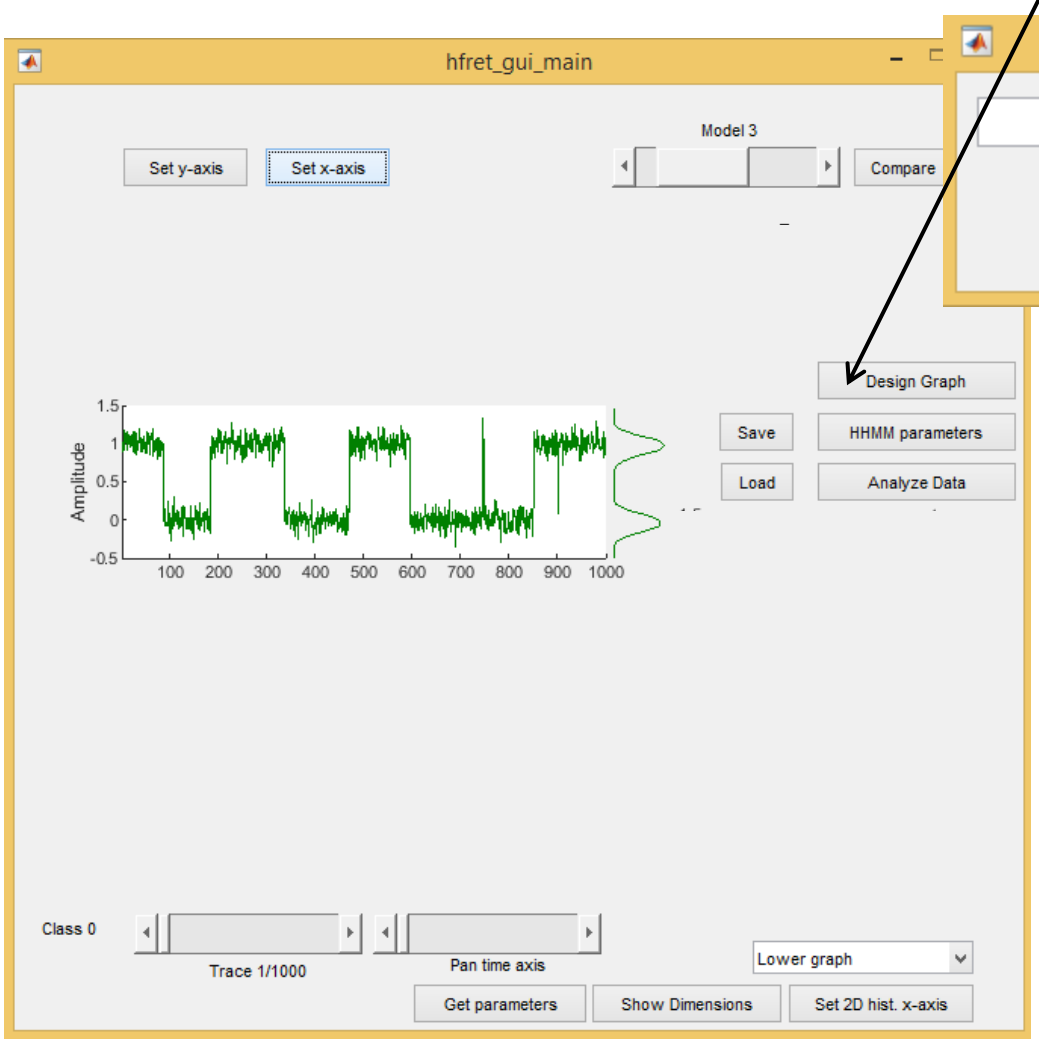
X axis adjustments

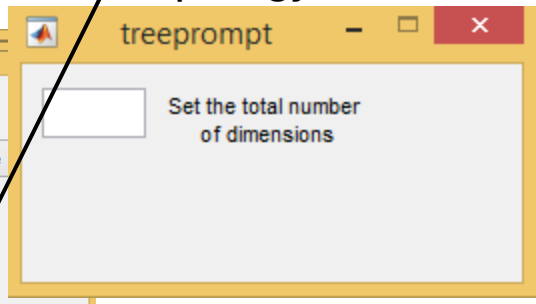




Time axis slider allows trajectory to be panned

Design Model Topology



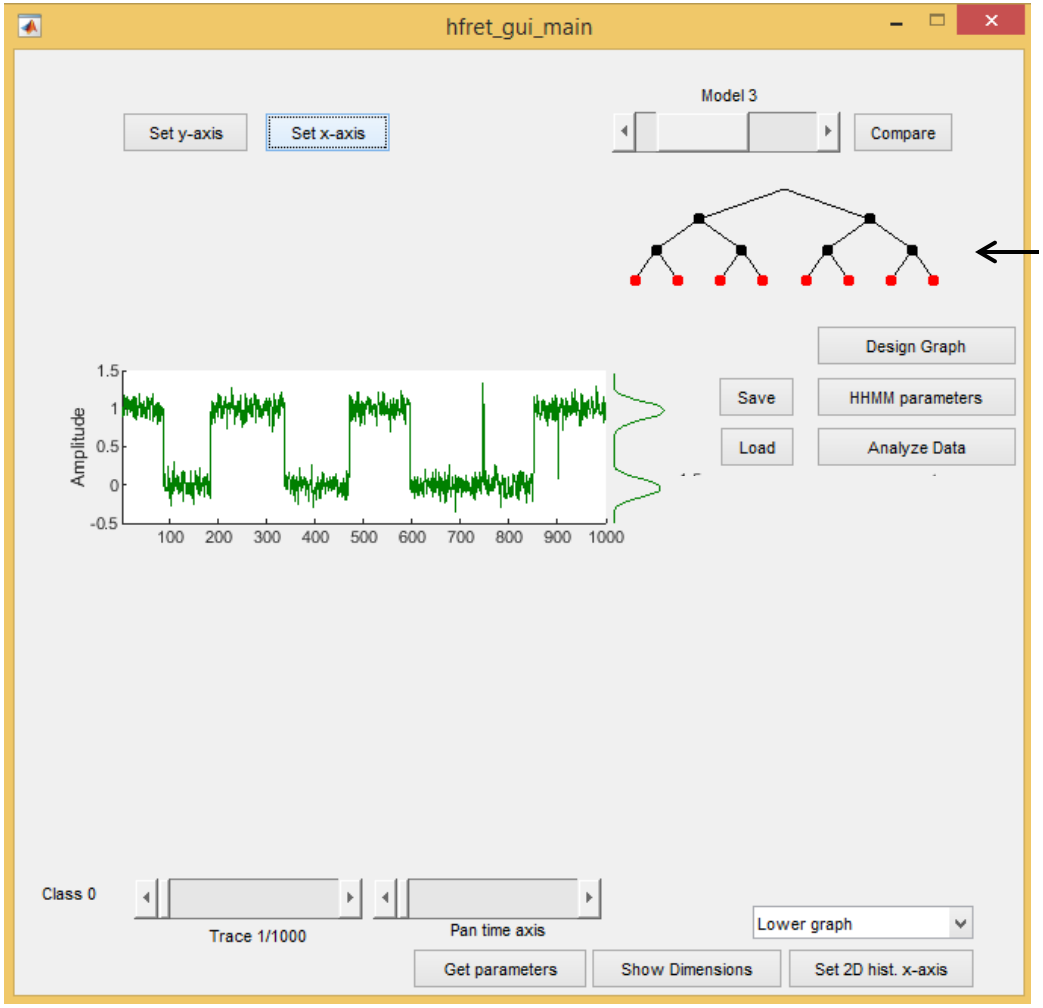


Prompts:

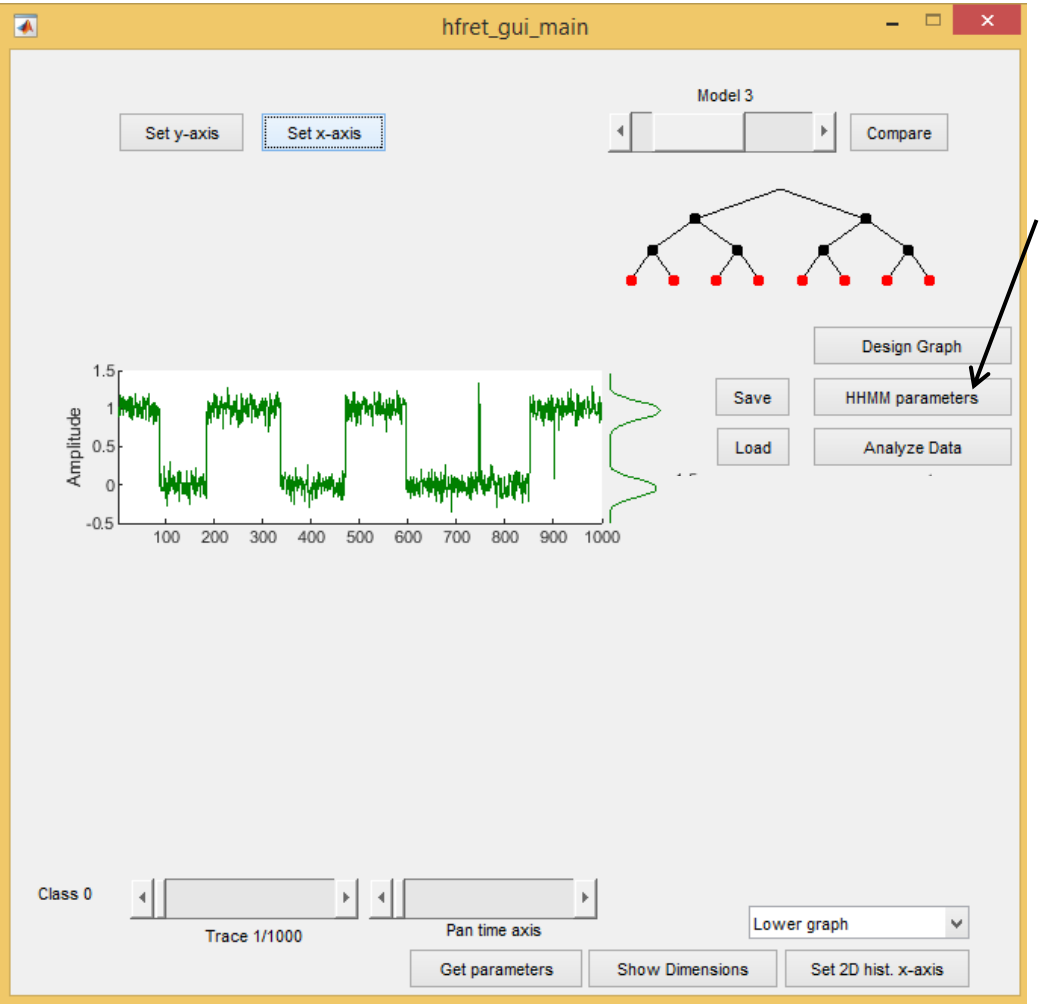
(1) Total # of dimensions, D

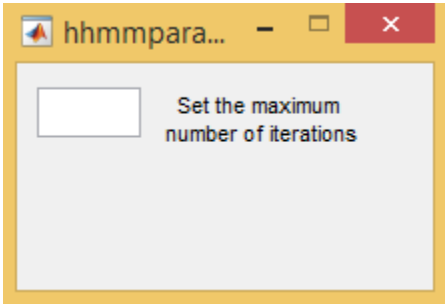
(2) # of distinct values at level d>1

(3) d=1, # of distinct values of the direct dimension



Model topology appears here. Shown: D=3, #=2 for all values of d.





Set algorithm control parameters

Prompts:

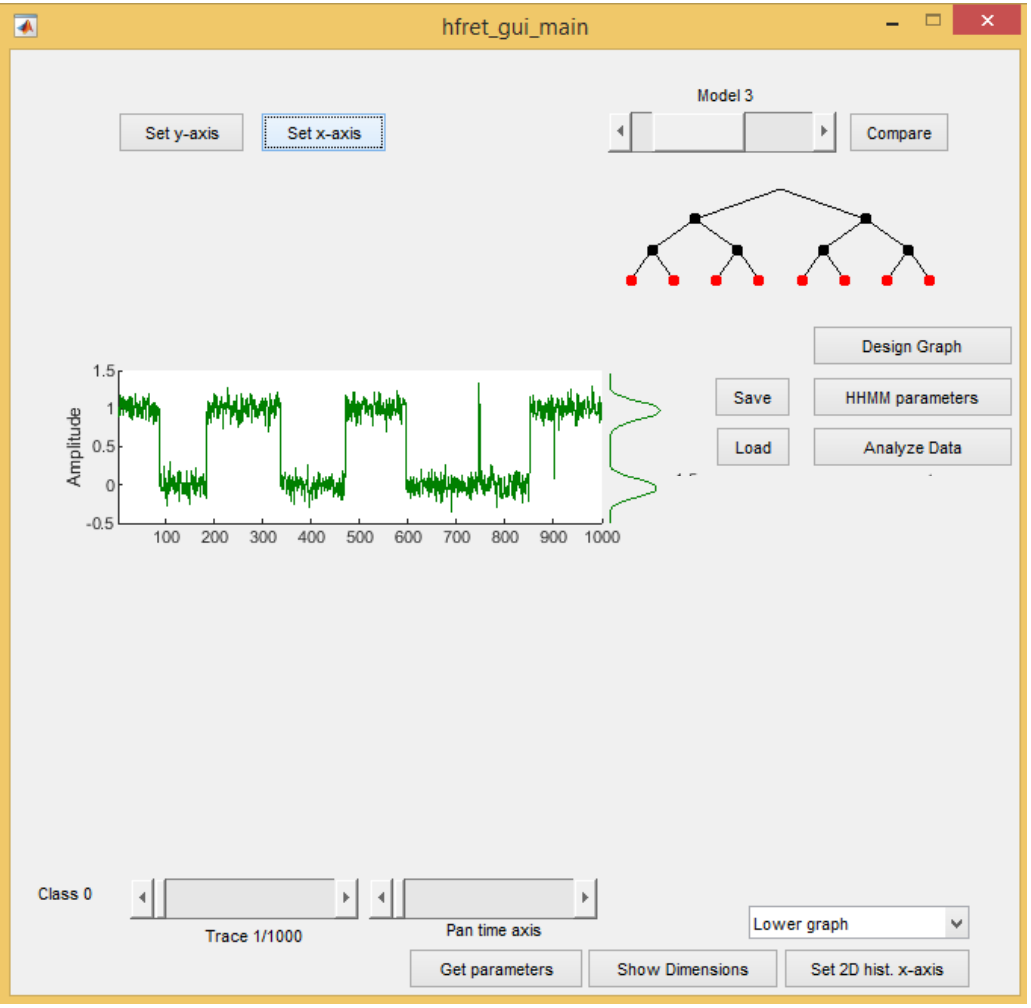
(1) Maximum number of iterations

(2) Number of restarts (kinetic parameters randomly drawn)

(3) Whether each trajectory has the same emission distribution (type “y” or “n”)

(4) Automatic or manual entry of normal distribution means(if “n,” the means are entered next; here, entering [0 1] would do)

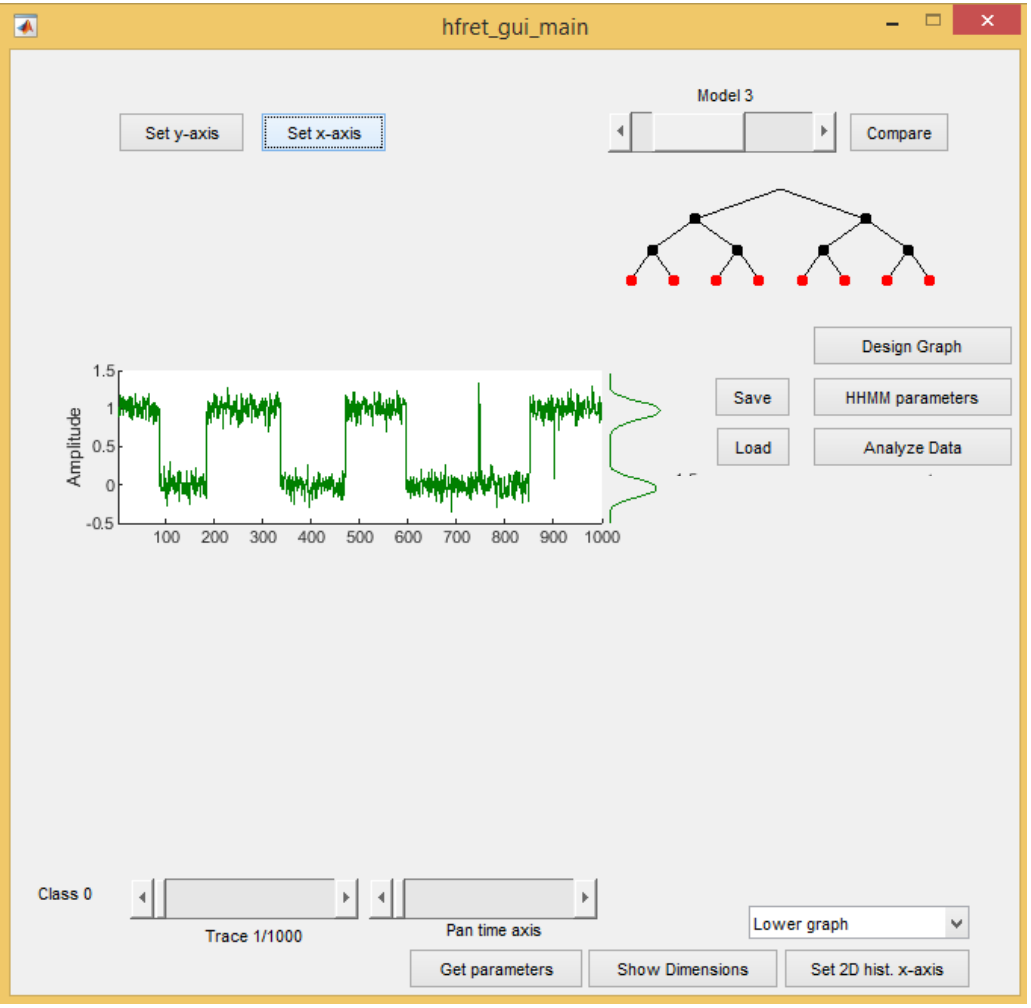
(5) Analysis of amplitudes or fractional amplitudes (if “n,” amplitude channel entered next; here, “1” would do”



Analyze data

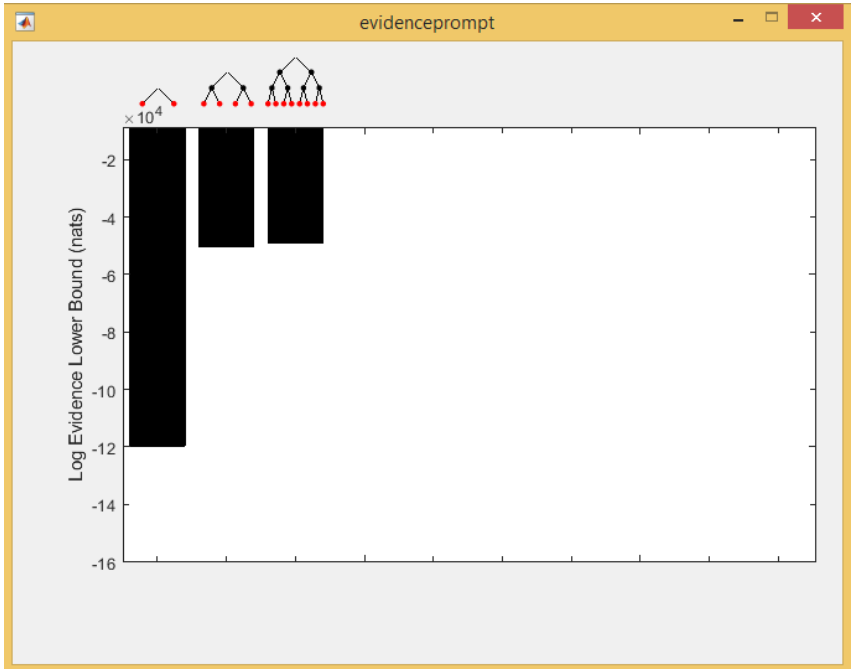
This can be a very time consuming step. Do not press other buttons while this occurs. A prompt in the command window will alert on completion.

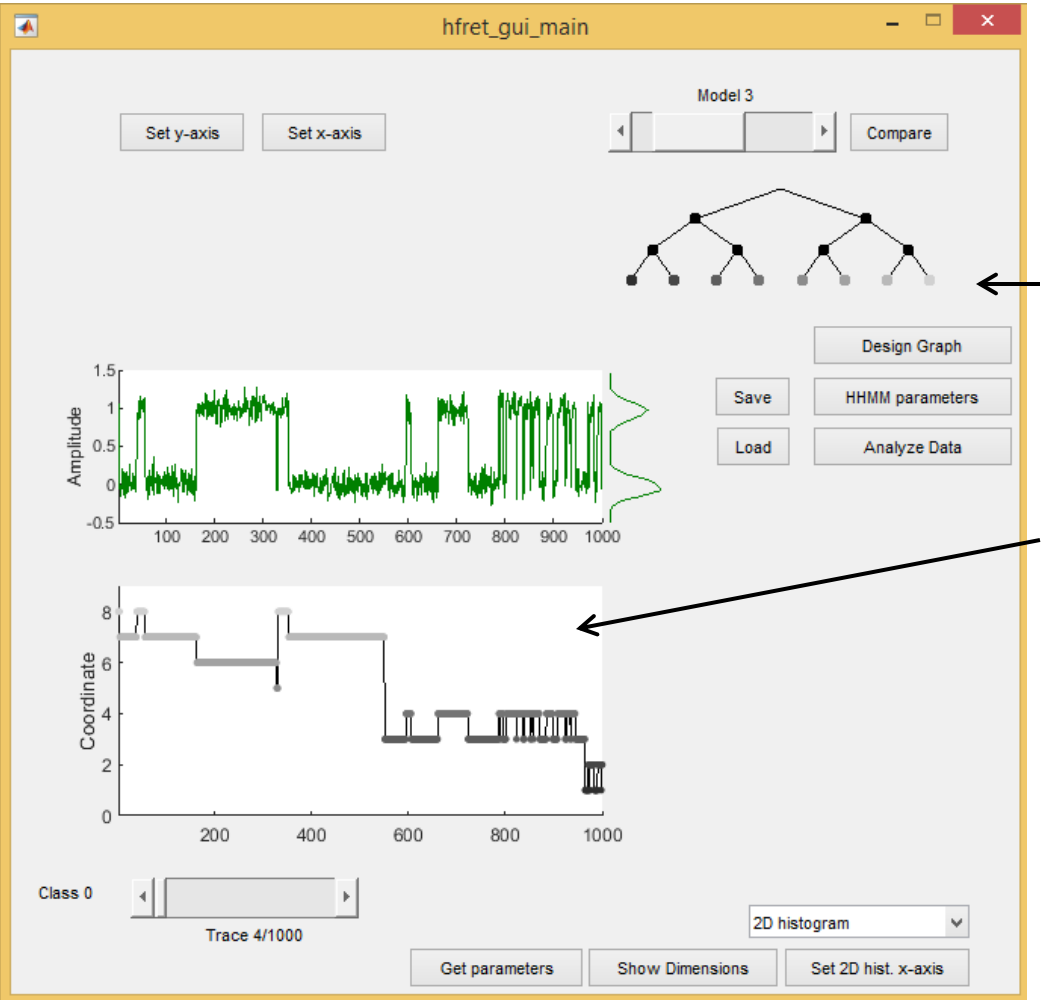
With two 3.6 GHz cores, shown model takes ~ 1 hr on 10^6 datapoints.



Can store up to 10 models in the gui session for comparison. Models are not stored until after analysis completes.

On pressing “Compare,” evidence lower bounds of the stored models are shown (as below.)

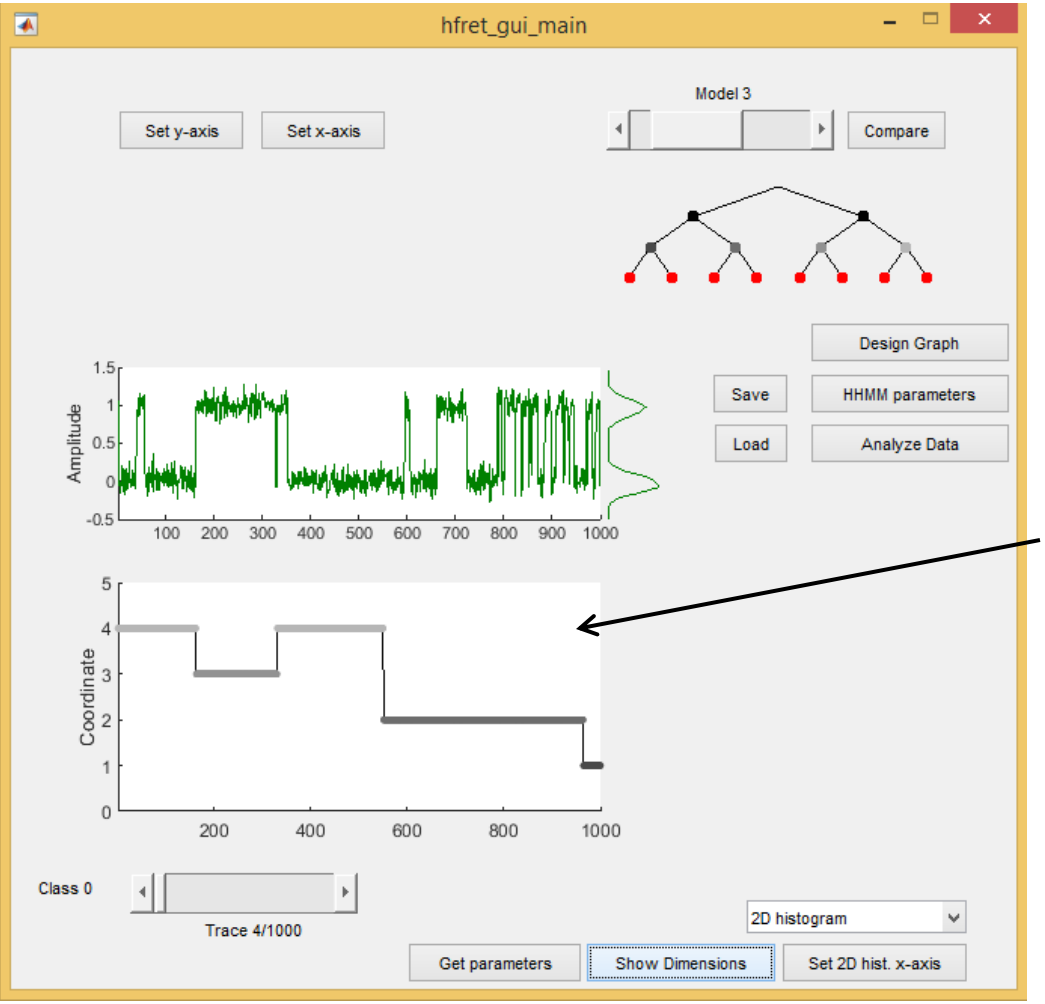




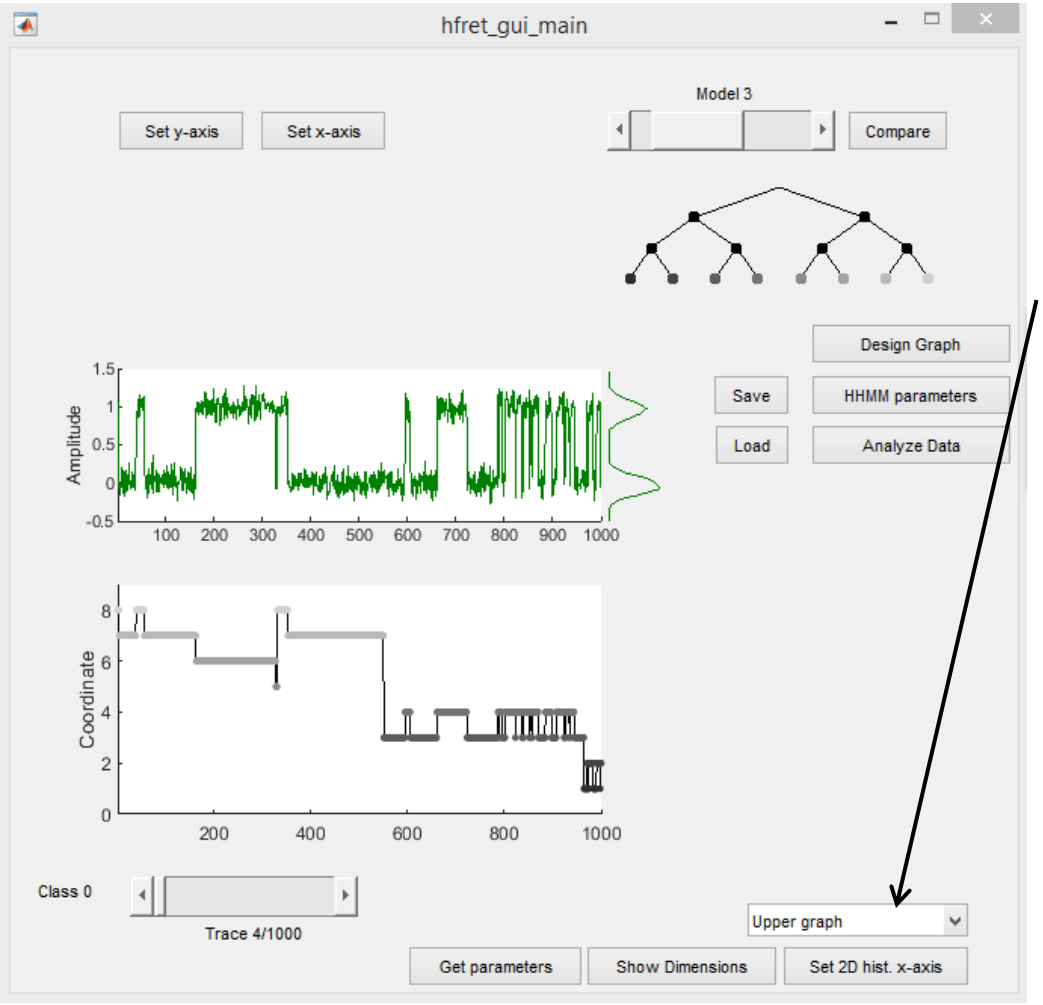
Click “Show dimensions.”

If non-direct representation is chosen, the level is indicated here by a gradient of grayscale. Here shown bottom level.

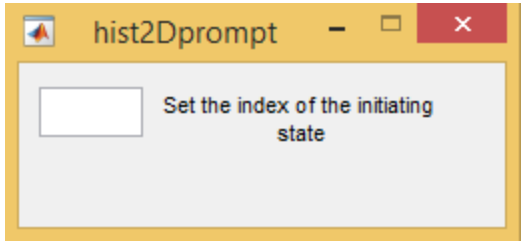
Values are both numbered (1-8) and color coded to correspond to the model topology diagram.



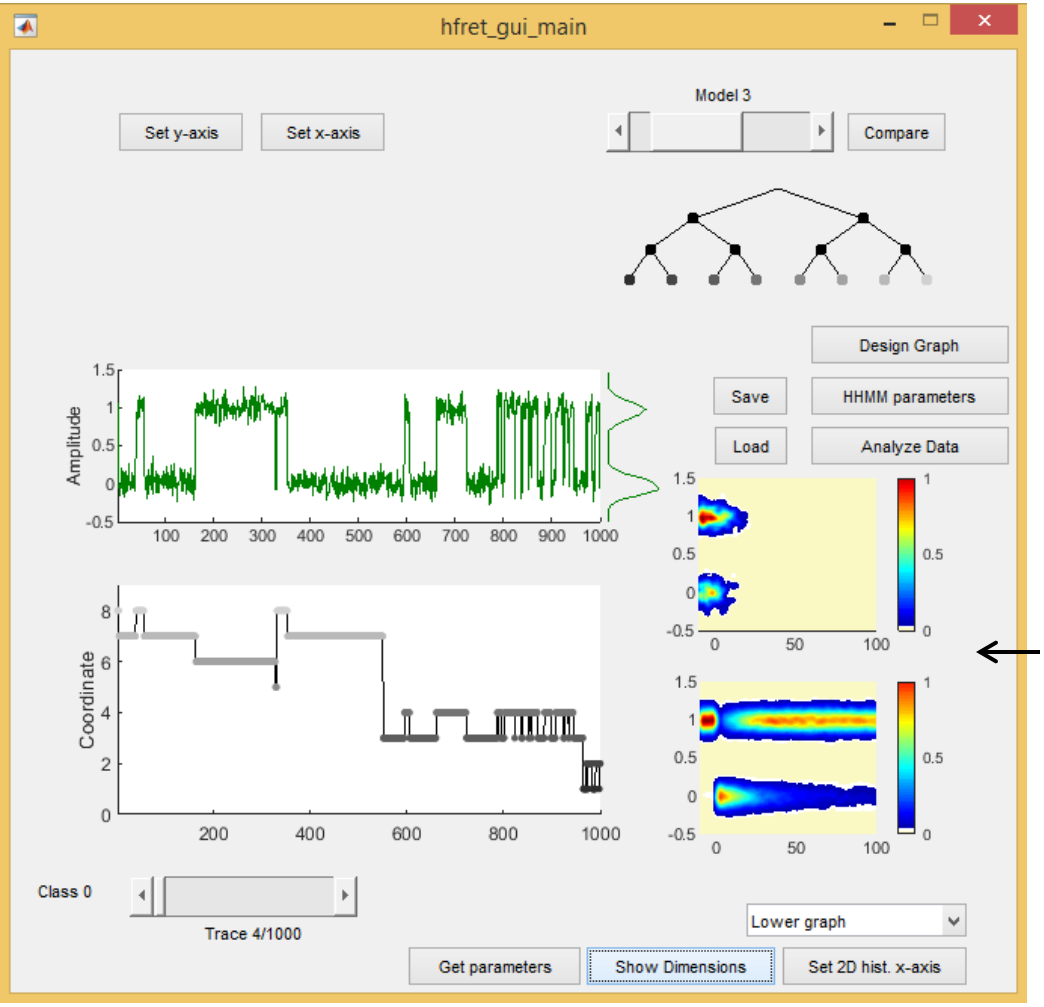
Intermediate level (d = 2) shown. Values are both numbered (1-4) and color coded to correspond to the model topology diagram.



For population plots (or 2D histograms), change to “Upper graph” or to “Lower graph”



Enter the initiating and then the terminating state index, which correspond to the index shown in the coordinate plot. The index always refers to the index at the bottom level.



Two graphs can be shown. They need not be from the same model.