

## User Manual

### Sequential Arrival

**Abstract:** This program classifies events that start and end sequentially. The events are divided into seven different categories depending upon their arrival and departure with respect to each other.

Type A: The 2<sup>nd</sup> color appears after the 1<sup>st</sup> color and departs after the 1<sup>st</sup> color.

Type B: The 2<sup>nd</sup> color appears after the 1<sup>st</sup> color but departs before the 1<sup>st</sup> color.

Type C: The 2<sup>nd</sup> color appears more than one time after the 1<sup>st</sup> color appearance. This type of event also has both A and B type.

Type S: Both colors appear and depart simultaneously for a very short time period.

Type M: The 2<sup>nd</sup> color appears after the 1<sup>st</sup> color has disappeared.

Type N: The 2<sup>nd</sup> color appears and departs before the 1<sup>st</sup> color has appeared.

Type F: Events occur with a long gap between each other and are not further analyzed.

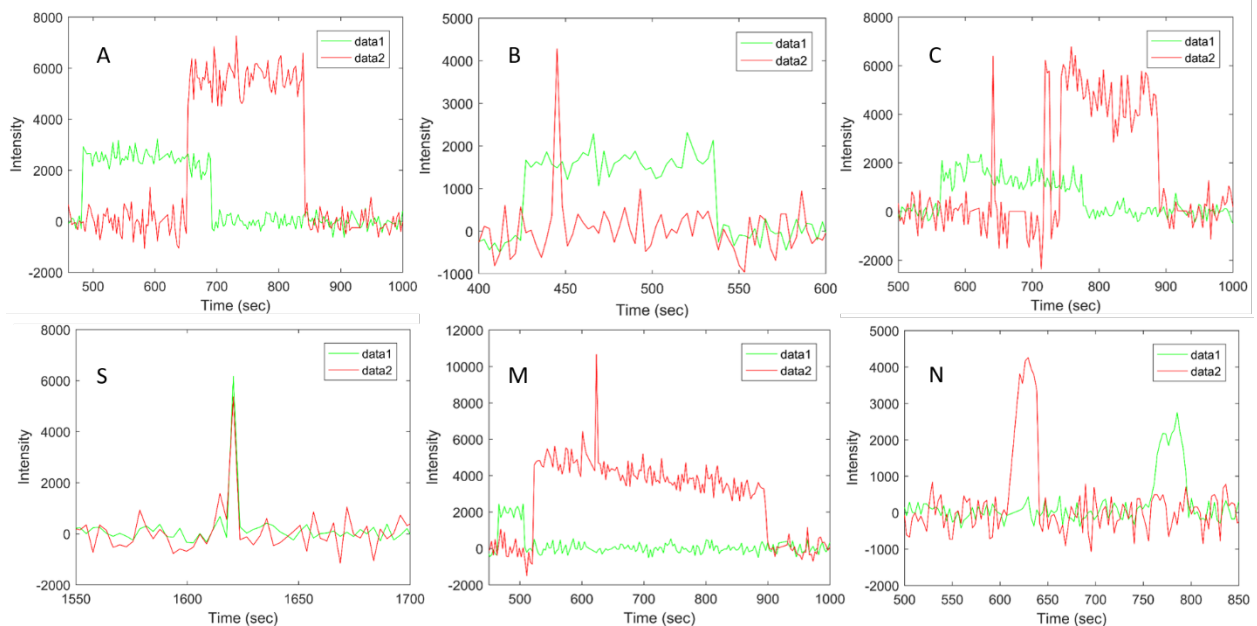


Figure 1 Different types of sequentially occurred events that are categorized based on their arrival and departure with respect to each other.

### Conditions on the start and end frames to classify the events:

- A type:  $f_{1s} < f_{2s} \ \&\& \ f_{1e} < f_{2e} \ \&\& \ f_{2s} < f_{1e}$   
B type:  $f_{1s} < f_{2s} \ \&\& \ f_{1e} > f_{2e}$   
C type: If Both A and B occurs  
S type:  $|f_{1s} - f_{2s}| < \text{delta1} \ \&\& \ |f_{1e} - f_{2e}| < \text{delta1} \ \&\& \ \Delta f_2 < \text{delta2} \ \&\& \ \Delta f_1 < \text{delta2}$   
M type:  $0 \leq f_{2s} - f_{1e} < \text{Limit}$   
N type:  $0 \leq f_{1s} - f_{2e} < \text{Limit}$   
F type:  $f_{2s} - f_{1e} \geq \text{Limit}$  or  $f_{1s} - f_{2e} \geq \text{Limit}$

where  $f_{1s}$  and  $f_{2s}$  are the start frames for 1<sup>st</sup> and 2<sup>nd</sup> color events respectively,  $f_{1e}$  and  $f_{2e}$  are the end frames for 1<sup>st</sup> and 2<sup>nd</sup> color events respectively.  $\Delta f_1$  and  $\Delta f_2$  are the frame intervals for the 1<sup>st</sup> color and 2<sup>nd</sup> color events respectively. The decision parameters,  $\text{delta1}$  is the cut-off value above which events will not be considered as simultaneous and  $\text{delta2}$  is the upper limit for the frame interval to be counted as short interaction (for S type of events). Limit is the user input value. If the difference between the start and end frames for both colors events is less than this input value, events will be classified as M or N. For the events with difference between the start and end frames larger than the Limit value are flagged and ignored.

### Definitions:

1.  $t_{rel\_start}$ , is the relative start time of the 2<sup>nd</sup> color with respect to the 1<sup>st</sup> color and is defines as,

$$t_{rel\_start} = t_s^2 - t_s^1$$

where  $t_s^1$  and  $t_s^2$  are the start times for the 1<sup>st</sup> and 2<sup>nd</sup> color events respectively.

2. Overlap time is defined as duration of the fluorescence intensity overlap. For A type of events, it is calculated as  $t_e^1 - t_s^2$ . For B and S type of events, it is assigned to 2<sup>nd</sup> color interval time. For M and N type overlap time is equal to zero.

3.  $t_{rel\_release}$ , is the relative release time of the 2<sup>nd</sup> color with respect to the 1<sup>st</sup> and is defined as,

$$t_{rel\_release} = t_e^2 - t_e^1$$

where  $t_e^1$  and  $t_e^2$  are the end times for the 1<sup>st</sup> and 2<sup>nd</sup> color events respectively.

4.  $t_{st2\_rel1}$ , is the difference between the arrival time of the 2<sup>nd</sup> color and the loss time of 1<sup>st</sup> color and is defined as,

$$t_{st2\_rel1} = t_s^2 - t_e^1$$

where  $t_s^2$  is the start time of the 2<sup>nd</sup> color, and  $t_e^1$  is the end time of the 1<sup>st</sup> color.

Algorithm: This program will first match the AOI number in both interval files. For each matched AOI, it will extract all the information about the high events (-3/1/+3) from CumulativeIntervalArray (cia) for both of the interval files (i.e. high events (-3/1/+3), start frame, end frame, delta frame, delta time, intensity, AOI number). It will also extract the time information corresponding to each frame for every AOI from AllTracesCellArray for both colors (which is the column 9 of AllTracesCellArray from the interval file). In order to account for different recording start times per color, time will be rescaled such that recording start times are zero. For every high value of the 1<sup>st</sup> color, program will test every high value of the 2<sup>nd</sup> color for the arrival and departure conditions. Depending upon the conditions satisfied by these events, it will classify events into different types and will calculate  $t_{rel\_start}$ , overlap,  $t_{rel\_release}$ , and  $t_{st2\_rel1}$  times. The program will return the results.mat and out.fig files in the output directory.

Instructions to run the code:

1. Click on the Two Colors Plot program and the following GUI will appear on the screen.

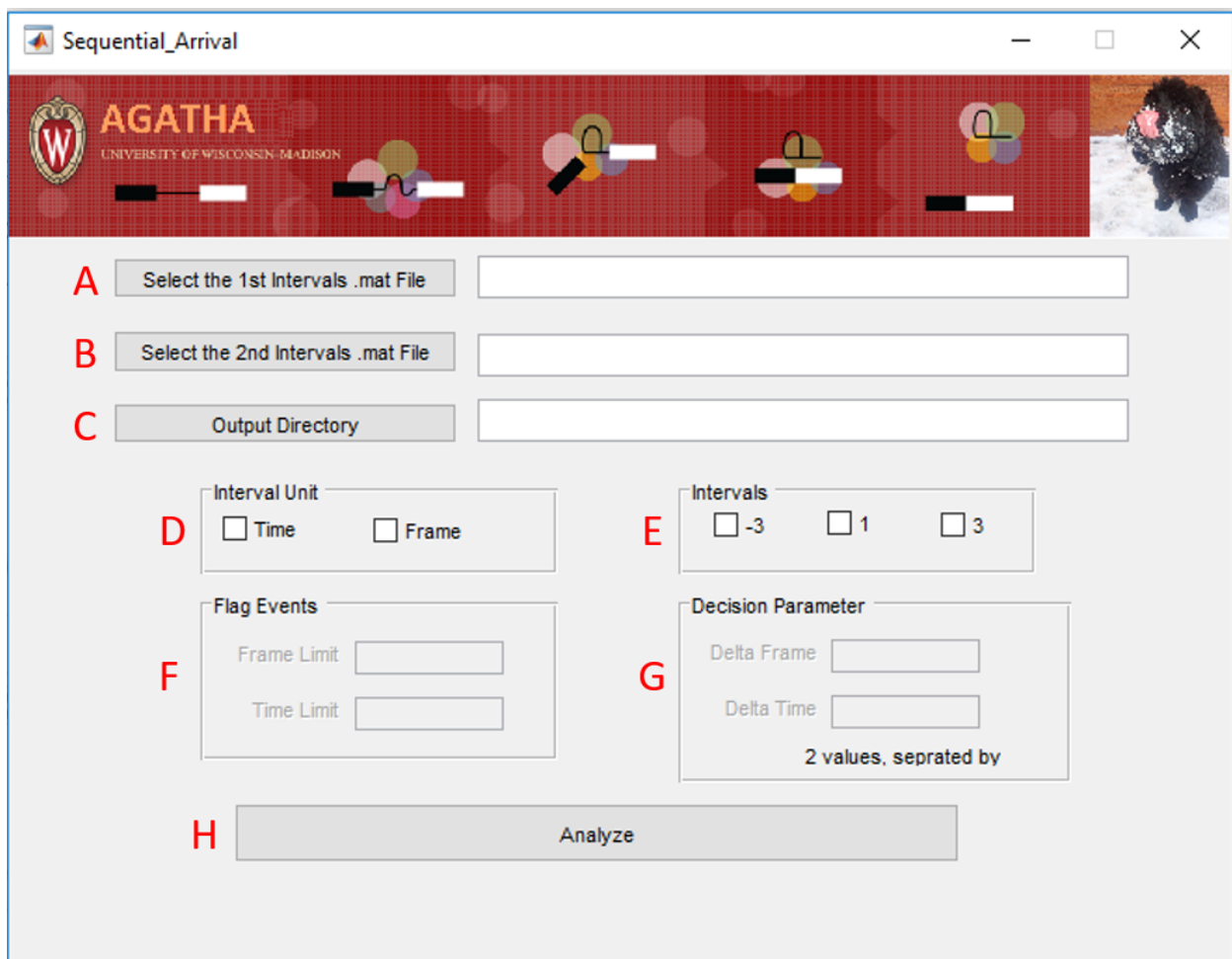


Figure 2 GUI for sequential arrival program.

2. Click on the Select the 1<sup>st</sup> intervals .mat file button to assign the 1<sup>st</sup> interval file (A in Fig. 3).

3. Click on the Select the 2<sup>nd</sup> intervals .mat file button to assign the 2<sup>nd</sup> interval file (B in Fig.3).

4. Click on the Output Directory button to assign an output location (C in Fig. 1).

5. Choose the Interval Unit (D in Fig.3).

Time: It will probe the conditions at the start and end time of the events for both colors to classify the events.

Frame: It will probe the conditions at the start and end frames of the events for both colors to classify the events.

6. Choose high events you want to analyze (-3/1/3, glimpse event classification) or any combinations of these high values (E in Fig.3).

7. Enter the Frame Limit or Time Limit value (keeping in mind the choice of the Interval Unit in D) in Flag events (F in Fig.3). Frame or Time Limit value corresponds to the difference between the start and end frames or times for both. For the events with difference between the start and end frames or times larger than the Limit value are flagged and ignored.

8. Enter the value for the decision parameters (*delta1 and delta2 separated by comma*) keeping in mind the choice of the Interval Unit (G in Fig.3).

9. Hit Analyze button (H in Fig.3).

10. The program will return the results.mat and out.fig files in the output directory. The result.mat file contains two structures Out and Time\_st\_vs\_rl, Final\_frame \_matrix, Final\_time \_matrix, A\_info, B\_info, M\_info, N\_info, and S\_info. The out.fig is the bar graph that plots the number for the different type of sequential events.

The structure Out contains eight fields with Description containing the header information about final\_decision. An example is given in the Fig. 3. Time\_st\_vs\_rl contains two fields with a Description containing the header information about time.

Final\_Time\_Matrix contains (1\_relative start time) (2\_Overlap time) (3\_relative release time) (4\_AOI) (5\_time interval of the 1<sup>st</sup> color) (6\_ time interval of the 2<sup>nd</sup> color) (7\_  $t_{st2\_rel1}$ ) for all type of events. This extracted information can be used for further analysis. Final\_Frame\_Matrix contains the same information but in frame units. In case, if the user is interested in the any particular type of events, all the extracted information is also sorted into A\_info, B\_info, C\_info, M\_info and N\_info matrices. These matrices contain (1\_AOI) (2\_relative start time) (3\_ Overlap time) (4\_relative release time) (5\_  $t_{st2\_rel1}$ ) (6\_ time interval of 1<sup>st</sup> color) (7\_ time interval of 2<sup>nd</sup> color).

Note: To perform all the analysis using these codes make sure you process the Interval analysis using Glimpseloadimscroll. Ensure that same AOIs are evaluated for all colors that will be analyzed with event classification.

1<sup>st</sup> Input file is assigned green color by default.

2<sup>nd</sup> Input file is assigned red color by default.

The screenshot shows the MATLAB environment with the 'Out' structure and its details. The 'Out' structure has 8 fields: Description, final\_decision, num\_A, num\_B, num\_AB, num\_S, num\_ME, and num\_N. The 'final\_decision' field is a 19x1 cell array containing information about 19 AOIs. The 'num\_A' through 'num\_N' fields are scalar values representing the number of events of each type.

The 'final\_decision' field is expanded, showing a 19x1 cell array. The first two rows are highlighted: Row 1 contains 'A' (Event Type), 65 (Relative Start frame/time), 2 (Overlap Frame/times), 79 (Relative release frame/time), 106 (AOI number), 68 (Frame/time intervals corresponding to the 1<sup>st</sup> color event of AOI 106, labeled as A), 82 (Frame/time intervals corresponding to the 2<sup>nd</sup> color event of AOI 106, labeled as A), and -2 (difference between the arrival frame/time of the 2<sup>nd</sup> color and the loss frame/time of 1<sup>st</sup> color). Row 2 contains 'A' (Event Type), 205.3750 (Relative Start frame/time), 6 (Overlap Frame/times), 247.3650 (Relative release frame/time), 106 (AOI number), 214.3780 (Frame/time intervals corresponding to the 1<sup>st</sup> color event of AOI 106, labeled as A), 256.3870 (Frame/time intervals corresponding to the 2<sup>nd</sup> color event of AOI 106, labeled as A), and -6 (difference between the arrival frame/time of the 2<sup>nd</sup> color and the loss frame/time of 1<sup>st</sup> color).

	1	2	3	4	5	6	7	8	
1	'A'	65	2	79	106	68	82	-2	Frame Information
2	'A'	205.3750	6	247.3650	106	214.3780	256.3870	-6	Time Information
3	Event Type	Relative Start frame/time	Overlap Frame/times	Relative release frame/time	AOI number	Frame/time intervals corresponding to the 1 <sup>st</sup> color event of AOI 106, labeled as A	Frame/time intervals corresponding to the 2 <sup>nd</sup> color event of AOI 106, labeled as A	t <sub>st2_rel1</sub>	
4	A type of								
5	event is								
6	detected in								
7	this AOI								
8									
9									
10									
11									

Figure 3. A brief description about the Out structure.