# User Manual
Simultaneous Arrival

Abstract: This program classifies the events that occur simultaneously and end either simultaneously or sequentially. The events are divided into five different categories depending upon their departure with respect to each other.

Type A: Both colors appear and depart simultaneously.

Type B: Both colors appear simultaneously but the 2nd color (2nd interval file to be analyzed) departs after the 1st color (1st interval file to be analyzed).

Type C: Both colors appear simultaneously but the 2nd color (2nd interval file to be analyzed) departs before the 1st color (1st interval file to be analyzed).

Type D: Both colors appear simultaneously and both retain their signals till end.
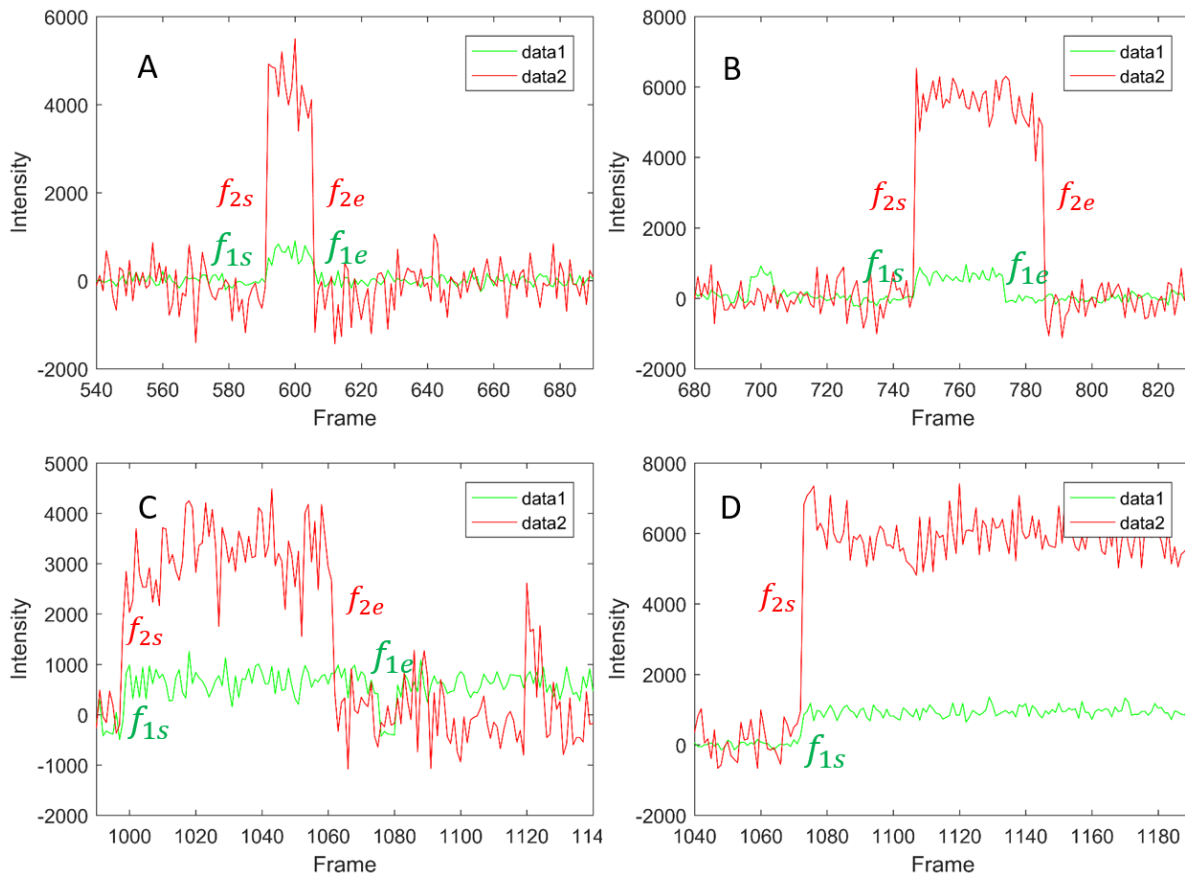
Type F: Events do not occur simultaneously and are not further analyzed.

Figure 1. Different types of simultaneously occurred events categorized based on their departure with respect to each other.

<u>Conditions on the start and end frames to classify the events</u>:

A type: $\quad |f_{1s} - f_{2s}| < delta\, f$ && $\;|f_{1e} - f_{2e}| < delta\, f$
B type: $\quad |f_{1s} - f_{2s}| < delta\, f$ && $\quad f_{2e} > f_{1e}$
C type: $\quad |f_{1s} - f_{2s}| < delta\, f$ && $\quad f_{2e} < f_{1e}$
D type: $\quad |f_{1s} - f_{2s}| < delta\, f$ && Glimpse Interval type = 3
F type: $\qquad f_{1s}\, \#\, f_{2s}$

where f_{1s} and f_{2s} are the start frames for 1st and 2nd color events respectively, and f_{1e} and f_{2e} are the end frames for 1st and 2nd color events respectively. The decision parameter, $delta\, f$ is the cut-off value above which events will not be considered as simultaneous.

<u>Definitions:</u>

1. Relative start time

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

where t_s^1 and t_s^2 are the start times for the 1st and 2nd color events respectively.

2. Overlap time

Overlap time is defined as duration of the fluorescence intensity overlap. For A, B and D type, it is assigned to 1st color interval time and for C type, it is assigned to 2nd color interval time.

Figure 2 Relative start time, Overlap time, and release time definitions.

3. Relative release time

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

where t_e^1 and t_e^2 are the end times for the 1st and 2nd color events respectively.

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
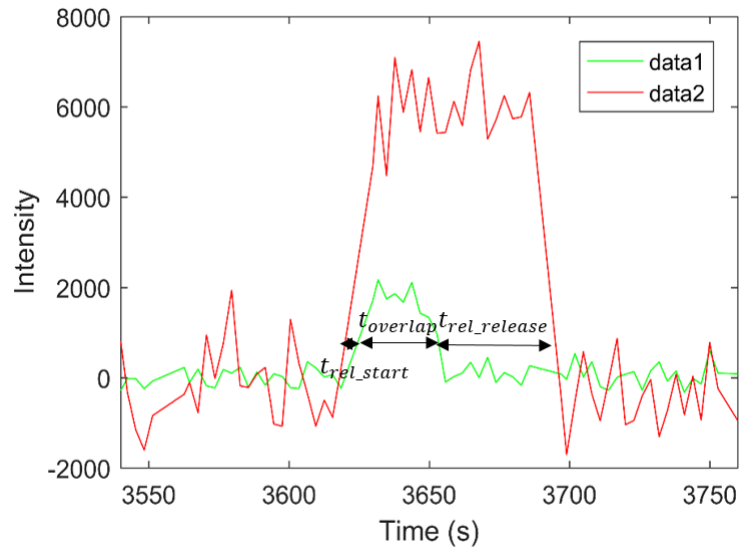
recording start times are zero. For every high value of the 1st color, program will test every high value of the 2nd color for the arrival and departure conditions. Depending upon the conditions satisfied by these events, it will classify events into the four types and will calculate the relative start, overlap, and relative release 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.
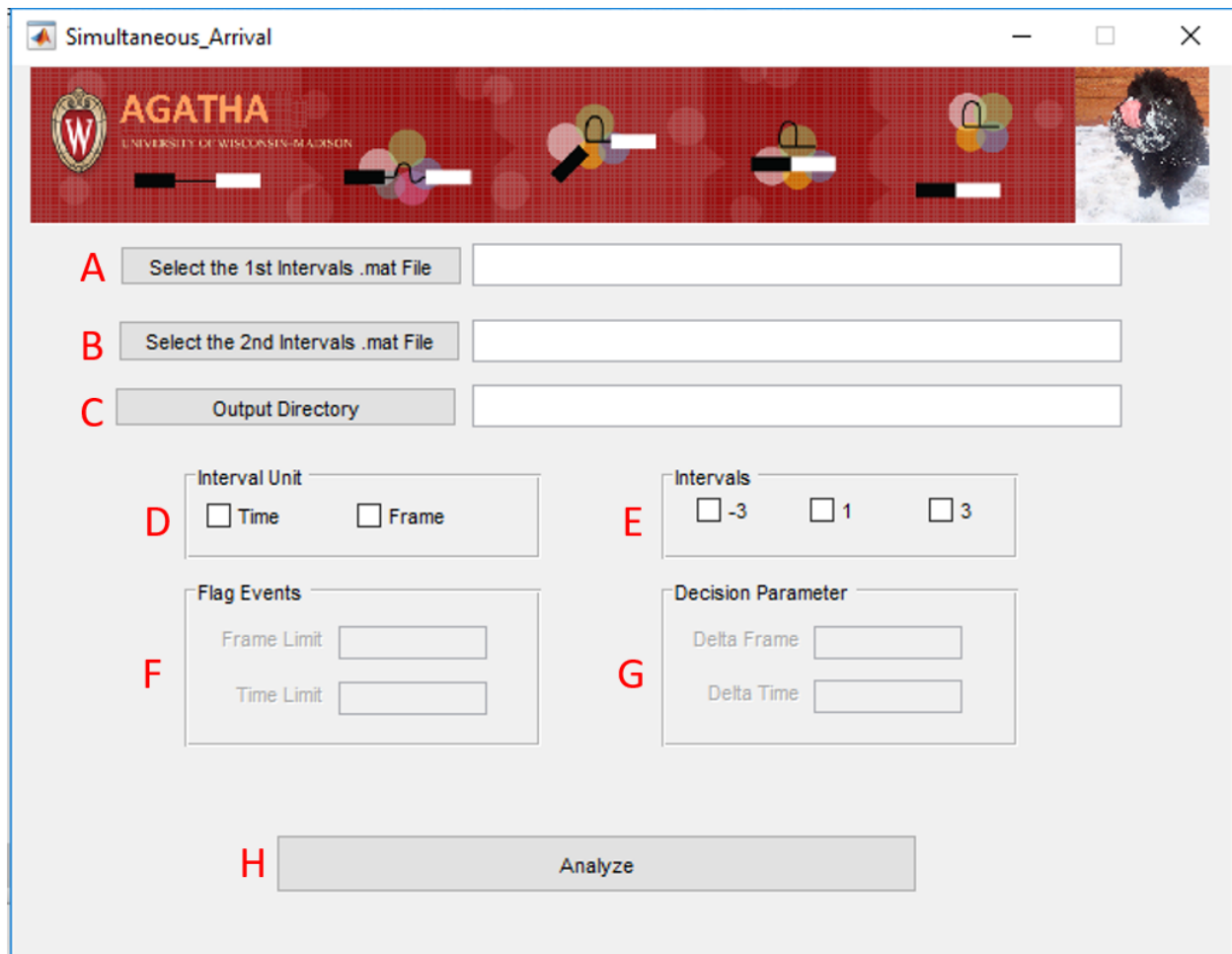

Figure 3 GUI for simultaneous arrival program.

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

3. Click on the Select the 2nd intervals .mat file button to assign the 2nd 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). If the difference between the start frames or start times for both colors events is less than this input value and more than decision parameter, those events are flagged. For the events with difference between the start frames or times larger than Frame or Time Limit value are not flagged and ignored.

8. Enter the value for the decision parameter ($delta\,f$) 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 a structure Out, Final_frame _matrix, Final_time _matrix, A_info, B_info, C_info, and D_info. The out.fig is the bar graph that plots the number for the different types of simultaneous events.

The structure Out contains seven fields with Description containing the header information about final_decision. An example is given in the Fig. 4. Final_Time_Matrix contains (1_relative start time) (2_Overlap time) (3_relative release time) (4_AOI) (5_ time interval of the 1st color) (6_ time interval of the 2nd color) 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, and D_info matrices. These matrices contain (1_AOI) (2_relative start time) (3_ Overlap time) (4_relative release time) (5_ time interval of 1st color) (6_ time interval of 2nd 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.

1st Input file is assigned green color by default.

2nd Input file is assigned red color by default

Editor - MultiState2MultiCia.m

Out ✕

1x1 struct with 7 fields

Field ▲		Value
{} Description	Array of 76 AOIs, Each cell contains information about the event type, AOI number and relative start and release times.	2x1 cell
{} final_decision		76x1 cell
num_A		48 Number of A type of events
num_B		64 Number of B type of events
num_C		4 Number of C type of events
num_F		10 Flagged events
num_D		2 Number of D type of events

Editor - MultiState2MultiCia.m

Out ✕ | Out.final_decision ✕ | Out.final_decision{1, 1} ✕

Out.final_decision{1, 1}

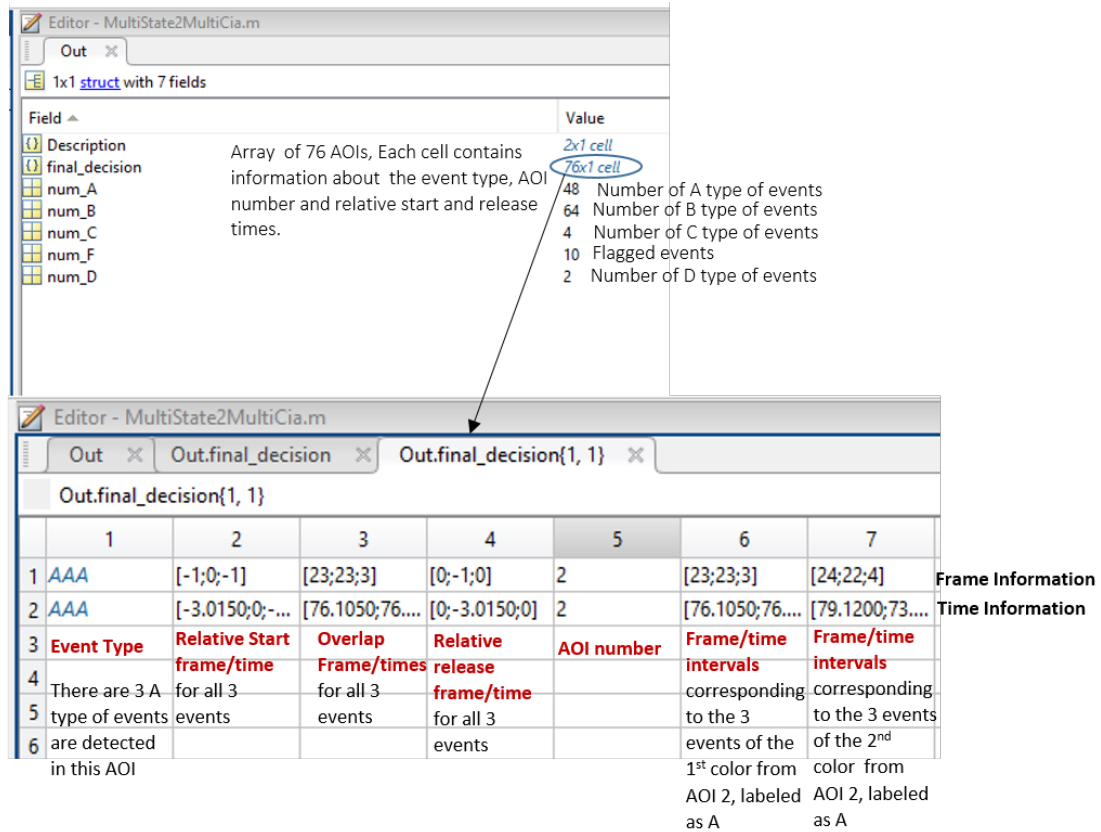|	1	2	3	4	5	6	7	
1	AAA	[-1;0;-1]	[23;23;3]	[0;-1;0]	2	[23;23;3]	[24;22;4]	Frame Information
2	AAA	[-3.0150;0;-...	[76.1050;76....	[0;-3.0150;0]	2	[76.1050;76....	[79.1200;73....	Time Information
3	Event Type	Relative Start frame/time	Overlap Frame/times	Relative release frame/time	AOI number	Frame/time intervals	Frame/time intervals	
4	There are 3 A type of events are detected in this AOI	for all 3 events	for all 3 events	for all 3 events		corresponding to the 3 events of the 1st color from AOI 2, labeled as A	corresponding to the 3 events of the 2nd color from AOI 2, labeled as A	
5								
6								

Figure 4. A brief description about the Out structure.