

# FlyGroomA Instructions v1.2

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## Video tutorials:

- [Building a param.csv file](#)
- [Processing text files with FlyGroomA](#)
- [Analyzing data with FlyGroomA](#)

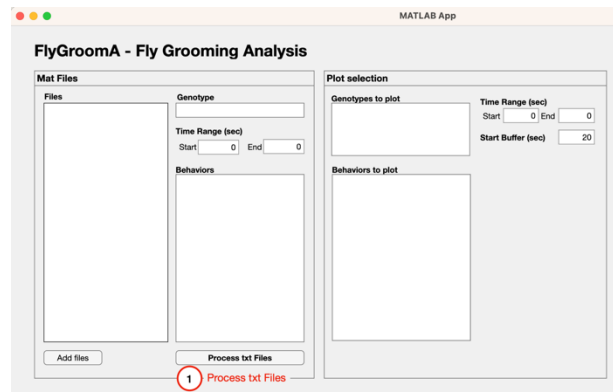
## Part 1 – Download, Install and Run

1. Git hub address: <https://github.com/hoopfere/FlyGroomA>
2. Click on “<> Code” button and select “Download Zip”
3. Open FlyGroomA/for\_redistribution/MyAppInstaller\_web file. If this file is not recognized by your computer, you’ll need to download the **R2022a** version of the Matlab Runtime application for Mac OS:  
<https://www.mathworks.com/products/compiler/matlab-runtime.html>. This download and installation takes a while. Restart your computer after installing
4. When you run the “MyAppInstaller\_web” file it should ask you to install FlyGroomA. Install to you Applications folder.
5. To run FlyGroomA, click on the FlyGroomA app in your applications folder.

## Part 2 – Processing text files

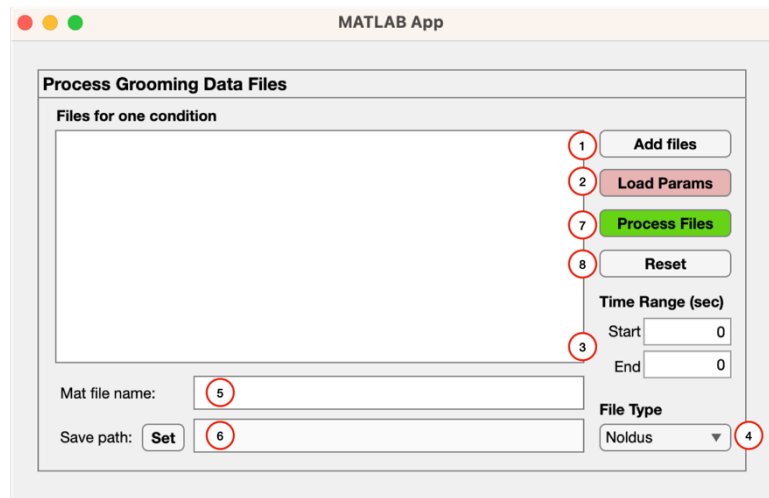
The first step in the analysis is to convert the txt files from your manual grooming program into mat files.

**Click on the “Process txt Files” button in the lower right of the “Mat Files” tab. This will open the “Process Grooming Data Files” window.**



1. Add files: select text files that correspond to experimental replicates from one experimental condition (e.g., the text files that correspond to separate flies of the same genotype run with the same experimental conditions).
2. Load Params: Select the params.csv file to use in processing

### Process text files



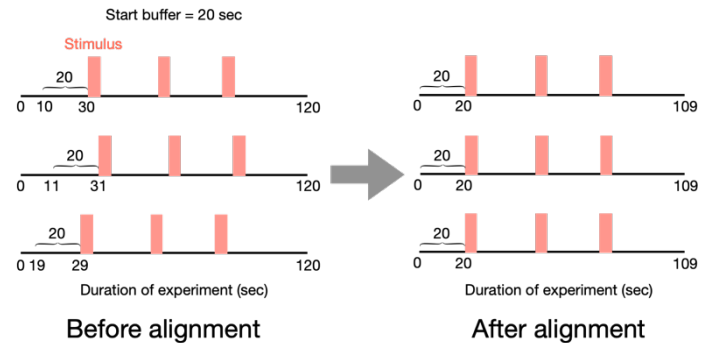
### Notes about param.csv files:

- Do not change the text of the column headers. This will break the code.
  - Each behavior should be assigned to only one class. To include a behavior in different classes, you will need to create separate params.csv for each instance.
  - The name of the behaviors must exactly match the names used in the text files, including capitalization.
  - Avoid the following characters: spaces, / \ . +
  - The name use used to indicate the stimulus must belong to “class\_stim”
3. Set the start and end times of the experiments. The duration should not exceed the minimum duration of any experiment. For example, if you have experiments that have durations of 120, 115, 130 second, you should set the end to 115 seconds.
  4. Select the filetype of the text files: Noldus or VCode.
  5. Type in name for mat file
  6. Select folder where you want to save mat file.
  7. Press “**Process Files**” button to create a mat file.
    - a. A window will appear notifying you when the mat file is created.
    - b. A warning may occur if the program finds behaviors in your text files that are not in the params.csv file. **This behavior will not be added to the mat file!** This error likely occurs because the name in the params file doesn’t exactly match what was used in the text files. Correcting the name in the params file should fix this issue.
  8. **Reset** will reset the following: text files, time range, mat file name. The params.csv file, save path and filetype will remain the same.
  9. When you are finished, close the window and return to the main FlyGroomA window.

## Part 2 – Analyzing grooming

### A. Add files and specify what to plot

1. Add mat files to analyze. Click on a file will display behaviors in the mat file.
2. Specify time range for plots.
3. Specify the start buffer. This specifies how many seconds before the first stimulus to start the analysis for each file. This is used to align different experiments to the same point in time, which is the start of the first stimulus.
4. Select which genotypes to plot.
5. Select which behaviors to plot.



### Add files and specify what to plot

MATLAB App

FlyGroomA - Fly Grooming Analysis

Mat Files

Files

Genotype

Time Range (sec)

Start 0 End 0

Behaviors

Add files

Process txt Files

Plot selection

Genotypes to plot

Behaviors to plot

Time Range (sec)

Start 0 End 0

Start Buffer (sec) 20

Boxplots Histograms Ethograms

Grouping Behavior Control None

Graph Box plot Statistics None

Metric Binning

☐ Bout Frequency Total

☐ Bout Duration

☐ Percent Time

☐ Total Time

☐ Plot Abs. Diff. from Control

☐ Update mat files

☐ Export data to Excel

Save Path: Set

Plot

### B. Boxplots

1. **Grouping:** determines how data is grouped on plots
  - a. Behavior: Each plot shows one behavior for each genotype
  - b. Genotype: Each plot shows multiple behaviors for one genotype
2. **Control:** Specify which groups to use as the control in statistical analyses. When a control is specified, posthoc comparisons will be done between the control and other groups (e.g., con vs genotype1, con vs genotype2). When no control is

specified, comparisons will be done between all groups (e.g., con vs genotype1, con vs genotype2, genotype 1 vs genotype 2).

3. **Metric:** Select all metrics to plot.
  - a. Bout Frequency: number of bouts per minute
  - b. Bout Duration: duration of bouts in seconds
  - c. Percent time: % time performing behavior
  - d. Total time: Cumulative time performing behavior.
4. **Statistics:** Select parametric or non-parametric statistics.
  - a. Parametric statistics:
    - i. Comparison of two groups: 2-tailed T-test
    - ii. Comparison of more than two groups: ANOVA + posthoc pairwise 2-tailed T-tests with Bonferroni correction for multiple comparisons
  - b. Non-parametric statistics:
    - i. Comparison of two groups: Mann-Whitney U-test (2 tailed)
    - ii. Comparison of more than two groups: Kruskal-Wallis + posthoc pairwise Mann-Whitney U-tests with Bonferroni correction for multiple comparisons.
  - c. Statistical results are included in Excel file (see below).
5. **Export data to Excel:** Writes out raw data and statistics for each plot into an excel file. Subplots will be put on separate sheets. The Excel file name will be Figure\_num\_Metric\_YearMonthDayHourMin.xls. For example, "Figure\_2\_Dur\_2404051222.xlsx" was from Figure 2 plot of Duration made on 4/5/24 at 12:22PM.
6. **Specify where to save excel file.**
7. **Plot!**

## Boxplots

**FlyGroomA - Fly Grooming Analysis**

**Mat Files**

Files: 26B12-24C08.mat, 77C10-26A02.mat, CON.mat

Genotype: 26B12-24C08

Time Range (sec): Start 0 End 120

**Behaviors**

abdominal\_cleaning, antennal\_cleaning, b\_1st\_and\_2nd\_leg\_rub, b\_2nd\_and\_3rd\_leg\_rub, back\_leg\_grooming\_attempt, back\_leg\_rubbing, backing\_up, body\_cleaning, eye, first\_leg\_notum, front\_leg\_rubbing, front\_proximal\_leg\_rub, genital\_cleaning

**Plot selection**

Genotypes to plot: 26B12-24C08, 77C10-26A02, CON

Behaviors to plot: abdominal\_cleaning, antennal\_cleaning, b\_1st\_and\_2nd\_leg\_rub, b\_2nd\_and\_3rd\_leg\_rub, back\_leg\_grooming\_attempt, back\_leg\_rubbing, backing\_up, body\_cleaning, class\_abdominal\_cleaning, class\_back\_leg\_rubbing, class\_front\_leg\_rubbing, class\_head\_cleaning, class\_other

Time Range (sec): Start 0 End 120

Start Buffer (sec): 20

**Boxplots** | Histograms | Ethograms

1. Grouping: Behavior

2. Control: None

3. Metric: Bout Frequency

4. Statistics: None

5. Export data to Excel: ☐

6. Save Path: Set

7. Plot

## C. Histograms

1. **Plot stimulus:** When checked this will add a shaded bar to regions of histogram that correspond to stimulus on. The stimuli will appear in the table below.
2. **Color of stimulus:** Specify this as Red, Green, Blue values between 0-1. The adjacent circle will turn that color.
3. **Transparency of stimulus:** Value must be between 0 (completely transparent) to 1 (completely opaque). Default = 0.25.
4. **Frame rate & Smoothing:**
  - a. Make sure the frame rate matches that of videos if different from 30 frames per second (fps).
  - b. **Smoothing:** The data in the histogram is smoothed with a running mean. This specifies the window of time over which to average the data. The default is 1 second, meaning the histogram will show the average fraction of flies performing a behavior in 1-second intervals.

## 5. Plot!

### Histogram

MATLAB App

#### FlyGroomA - Fly Grooming Analysis

**Mat Files**  
**Files**  
26B12-24C08.mat  
77C10-26A02.mat  
CON.mat  
  
**Genotype**  
26B12-24C08  
  
**Time Range (sec)**  
Start 0 End 120  
  
**Behaviors**  
abdominal\_cleaning  
antennal\_cleaning  
b\_1st\_and\_2nd\_leg\_rub  
b\_2nd\_and\_3rd\_leg\_rub  
back\_leg\_grooming\_attempt  
back\_leg\_rubbing  
backing\_up  
body\_cleaning  
eye  
first\_leg\_notum  
front\_leg\_rubbing  
front\_proximal\_leg\_rub  
genital\_cleaning  
  
Add files Process txt Files

**Plot selection**  
**Genotypes to plot**  
26B12-24C08  
77C10-26A02  
CON  
  
**Time Range (sec)**  
Start 0 End 120  
  
**Start Buffer (sec)** 20  
  
**Behaviors to plot**  
abdominal\_cleaning  
antennal\_cleaning  
b\_1st\_and\_2nd\_leg\_rub  
b\_2nd\_and\_3rd\_leg\_rub  
back\_leg\_grooming\_attempt  
back\_leg\_rubbing  
backing\_up  
body\_cleaning  
class\_abdominal\_cleaning  
class\_back\_leg\_rubbing  
class\_front\_leg\_rubbing  
class\_head\_cleaning  
class\_other

**Boxplots** **Histograms** **Ethograms**  
  
**Stimulus**  
☒ Plot Stimulus  
**Stimulus times (sec)**

Start	End	Durat
20	25	
55	60	
90	95	

  
**Stimulus Color**  
R G B T  
1 0.5 0.5 0.25  
  
Frame Rate (fps) 30  
Smoothing (sec) 1  
  
**Plot**

## D. Ethograms

1. Ethogram type: Currently, only plots “Compact” which creates a raster plot of selected behaviors. Each line is a separate fly. When multiple genotypes are selected, one ethogram per genotype is created.
2. Plot Stimulus: Check to add a shaded bar indicating stimulation times. Color can be specified by RGB.
3. Plot!

## Ethograms

MATLAB App

### FlyGroomA - Fly Grooming Analysis

**Mat Files**

**Files**

- 26B12-24C08.mat
- 77C10-26A02.mat
- CON.mat

**Genotype**

26B12-24C08

**Time Range (sec)**

Start: 0 End: 120

**Behaviors**

- abdominal\_cleaning
- antennal\_cleaning
- b\_1st\_and\_2nd\_leg\_rub
- b\_2nd\_and\_3rd\_leg\_rub
- back\_leg\_grooming\_attempt
- back\_leg\_rubbing
- backing\_up
- body\_cleaning
- eye
- first\_leg\_notum
- front\_leg\_rubbing
- front\_proximal\_leg\_rub
- genital\_cleaning

Add files

Process txt Files

**Plot selection**

**Genotypes to plot**

- 26B12-24C08
- 77C10-26A02
- CON

**Time Range (sec)**

Start: 0 End: 120

**Start Buffer (sec)**

20

**Behaviors to plot**

- abdominal\_cleaning
- antennal\_cleaning
- b\_1st\_and\_2nd\_leg\_rub
- b\_2nd\_and\_3rd\_leg\_rub
- back\_leg\_grooming\_attempt
- back\_leg\_rubbing
- backing\_up
- body\_cleaning
- class\_abdominal\_cleaning
- class\_back\_leg\_rubbing
- class\_front\_leg\_rubbing
- class\_head\_cleaning
- class\_other

Boxplots Histograms Ethograms

Ethogram type: Compact 1

☒ Plot Stimulus

**Stimulus Color**

R	G	B
1	0.5	0.5

2

3 Plot