Getting started with *SleepMat*

This walkthrough is an introduction to the use of SleepMat software to analyze the sleep and circadian behavior of Drosophila.

Installation procedure

Mac installation

Go to the Github repository https://github.com/shijusisobhan/SleepMat2022.1

1. Download the *SleepMat_satndalone_app_mac.app.zip* file
2. Extract the file SleepMat
3. Double click on the extracted file. You may see the following error message
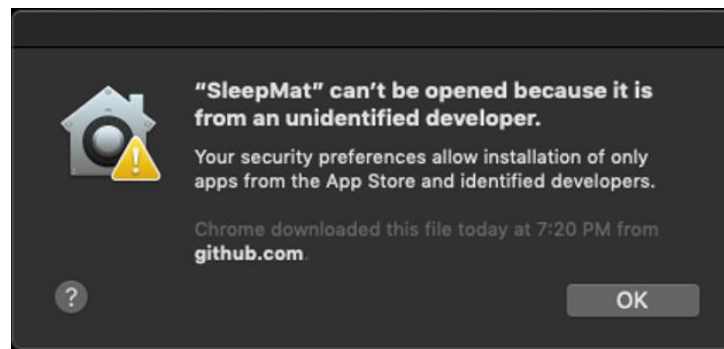

4. Go to system preference, click on security & privacy, click on General then click on app store and identified developers

5. again try with double click on the SleepMat. If you again see an error message, then again go to system preference, click on security & privacy, click on General and click on open anyway
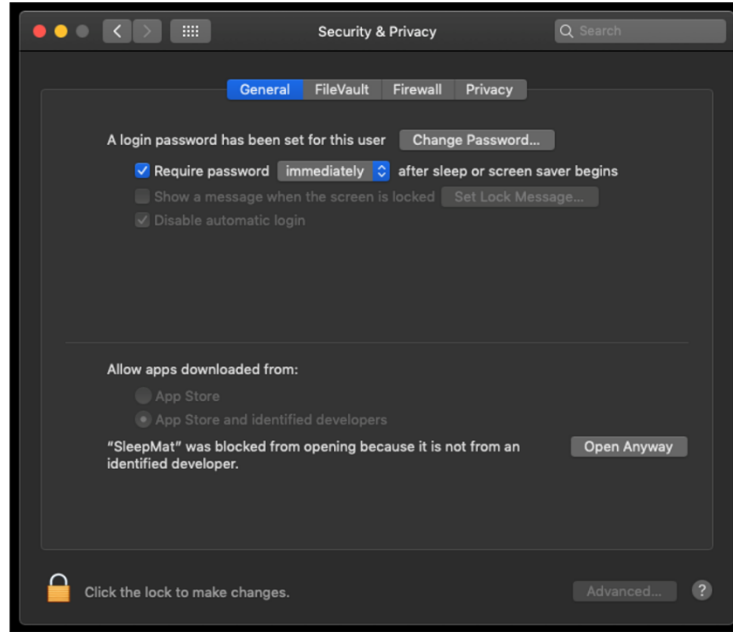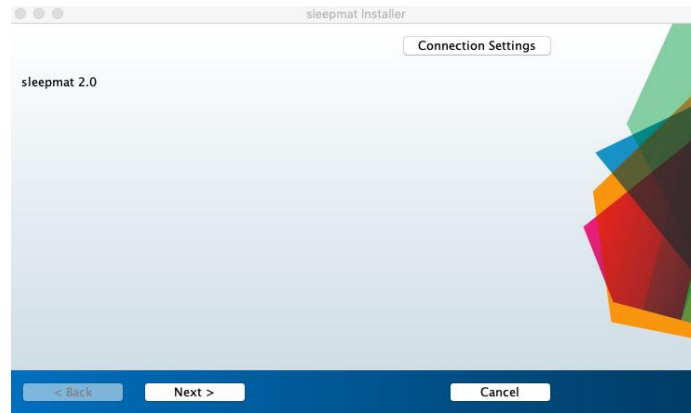
6. Again double click on the SleepMat

Note: If all the above steps failed, then copy the *sleepmat* file to an USB or external hard disk. Then repeat the step 5 & 6
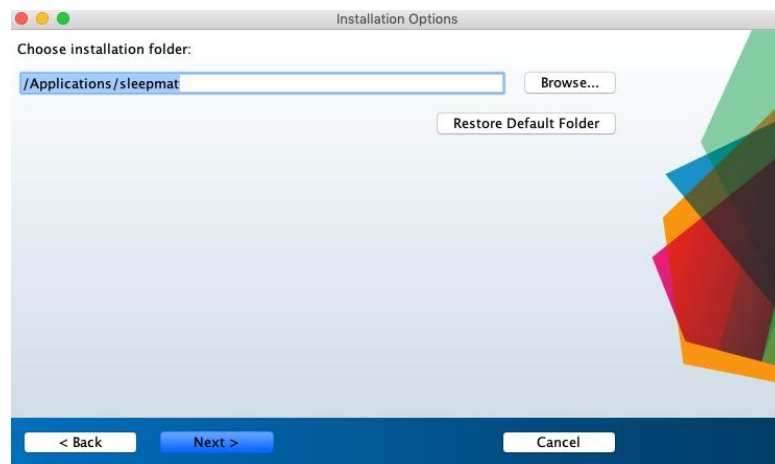
7. Enter your device password
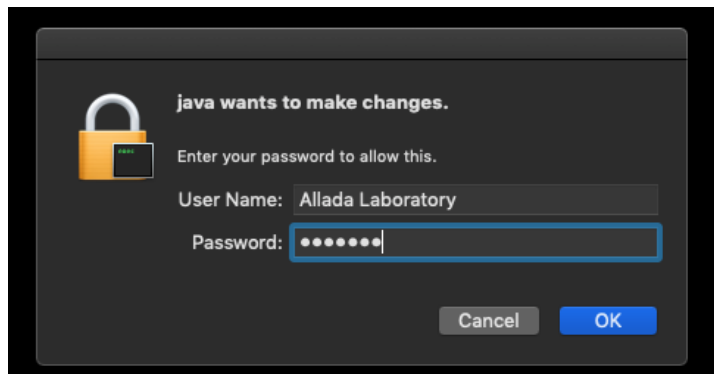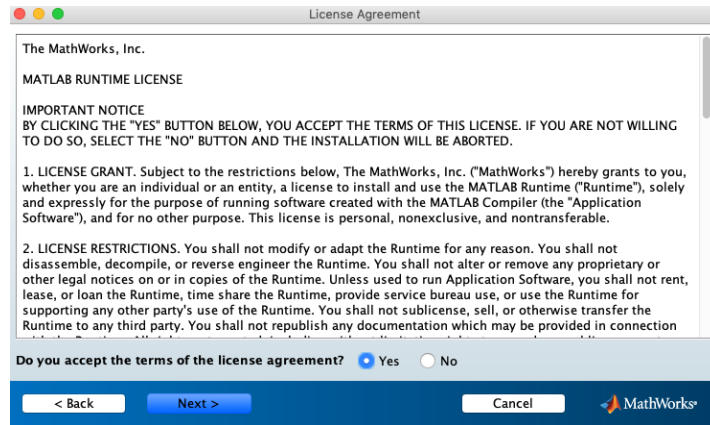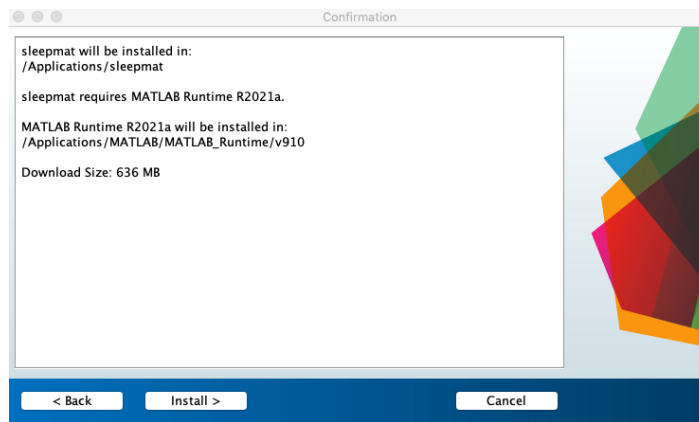

8. Click Next
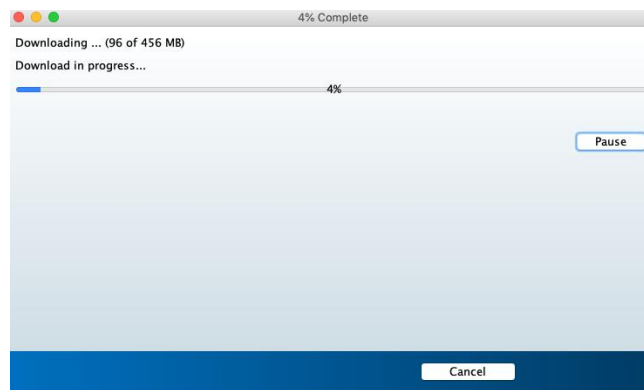
9. Click Next

9. Click Next
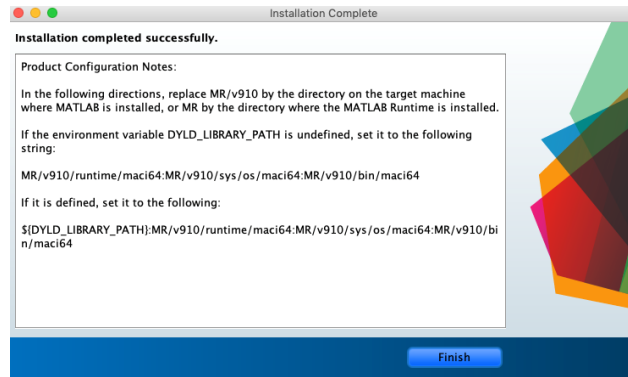

9. check Yes and click Next

9. Click on install

9. Wait until finish the installation procedure

10. Click on Finish

10. go to /Applications/sleepmat/application, right click on sleepmat, click on Make Alias.

11. Click enter and drag the alias to your desktop.

12. Double click on the sleepmat icon to open the app

Installation on Windows

1. Download the *SleepMat_standalone_app_Windows.exe* file
2. Double click on the *SleepMat_standalone_app_Windows.exe* file.
3. Please check the box for creating the shortcut to the Desktop.
4. Depending on your machine and internet connection it will take 5-10 min to complete the installation. By default, SleepMat installed in the 'C:\Program Files' folder
5. To run the *SleepMat*, double click on the *SleepMat* icon on the desktop

Run *SleepMat* without Installation

Pre-requisite: MATLB to be installed in your PC or Mac. Statistical toolbox also required to run the program successfully. *SleepMat* matlab p-code is developed in MATLAB version 2019a on a Windows platform and modified for the Mac operating system. Both Windows and Mac versions also function well with the MATLAB 2021b version.

1. Download the *sleepmat_Windows_require_MATLAB.zip*   OR
sleepmat_mac_require_MATLAB.zip

2. Extract the files

3. Open MATLAB

4. Change the current folder to the new folder where *SleepMat* software is located.

E.g., If *SleepMat* software is saved in the 'D' drive, then to change the current folder, type

the following line in the MATLAB command window:

```
>> cd D:\sleepmat_Windows_require_MATLAB
>> |
```

5. To open the software, type 'sleepmat' in the MATLAB command window, then hit enter.
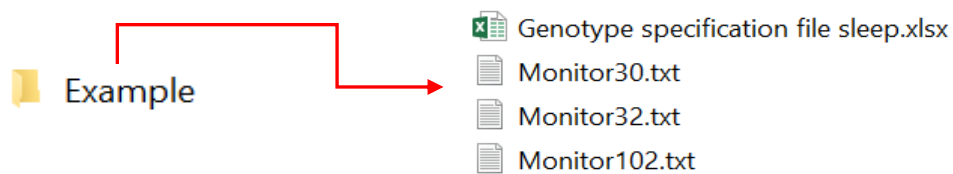
```
>> cd D:\sleepmat_Windows_require_MATLAB
>> sleepmat
>>
```

Walkthrough Example

This walkthrough is based on data submitted on the

https://github.com/shijusisobhan/SleepMat2022.1 Download the zip folder 'Example.zip', unzip

it and extract the files. Open the folder, now the folder should look like this:

The folder contains monitor files and a genotype specification file which describes the experiment, genotype, monitor files, channel number, and dates. Monitor file number 30 and 32 have data starting from *20 Jun 19 08:50:00* and ending at *3 Jul 19 10:57:00.* Monitor file 102 have data starting at 11 Jan 18 23:58:00 and ending at *17 Jan 18 15:00:00.*

Create a genotype specification file

Creating a genotype specification file is the most important, and time-consuming step in the *SleepMat* analysis. Here already we provide a genotype specification file (*genotype specification file sleep.xlsx*) and explain below how it is prepared.

- Open an excel file

- column A (Mandatory) : Here user has to specify the run number. The first cell (A1) is the header and A2 onwards are the run numbers. If you don't know about the run number, then enter some arbitrary numeric/alpha numeric value there (don't leave it blank).

- Column B (Mandatory): This is the monitor number and must be a numeric value. In this example, if we want to analyze the files 'Monitor30.txt', then you should enter 30 in this column.

- Column C (Mandatory): This is the genotype name, and it can be either alpha numeric or plain text (Don't use the characters (/, \, <, >, .) for naming the genotype which affects figures). In this example we have 3 genotypes, where *perS stock* flies are loaded on the both monitor 30 and 32.

- Column D, E (Mandatory): These are the range of channels you need to analyze. Column D is the start channel and column E is the end channel. These are numeric values and should be between 1 and 32.

- Column F (Mandatory): This is the start date of analysis and its format is the same as that appears in the monitor file (eg: **22 Jun 19**). It must be plain text. Start date doesn't mean that it is the actual starting date of Monitor file. It is user's choice. However, there is some rules to select the start date.

　　　　1) The start date should be present in the Monitor file.

2) light on time specified in the GUI should be present in the start date.

3) Start date is a reference date to analyze the data. If the user specifies a start date in the column F, every other day specified in the GUI should be with respect to this date.

4) It must be plain text and the format is same as that appears in the monitor file (22 Jun 19), not in a date format (22/08/19)

In this walkthrough example, Montor30.txt have data starting from *20 Jun 19 08:50:00.* First two days of data are redundant and we don't want those data. So here we interested only the data from *22 Jun 19* onwards. So, we specify the start date is *22 Jun 19* in the column F. Also, in this example analysis our light on time is 8 AM, which is available in the monitor file (Rule 1 & 2). So, when you analyze the Monitor file 30, Day-1 is 22 Jun 19. The date appeares in the monitor file 30 is, 22 Jun 19. So, you have to enter the date in that format. If the date is appeared in the Monitor file as 22-Jun-19, you should enter it in that format (plain text).

- Column G (Optional): This is the triage date, and it is optional. If you wish to evaluate triage conditions (conditions for determining whether the fly isdead or not) on a specific date, then you can enter the dates here. Date format is the same as that appears in the monitor file (eg: **30 Jun 19**). If this column is left blank, by default the software will evaluate triage conditions using the last day of analysis.

After completing the genotype specification excel file, save it as .xlsx file with any file name.

A	B	C	D	E	F	G	H
Run name	Monitor	Geno type	start channel	end channel	start dates	Triage date	
2970	30	perS stock	25	32	22 Jun 19	30 Jun 19	
2970	32	perS stock	1	4	22 Jun 19	30 Jun 19	
2970	32	w-iso 31 stock	17	28	22 Jun 19	30 Jun 19	
2615	102	per01 female_SD	1	32	13 Jan 18	16 Jan 18	

Example genotype specification file format

GUI

Before opening the GUI, make sure that your genotype specification file and all the monitor files are in the same folder.

- Open the *SleepMat* GUI

General Input

1. **Open file**: Click the 'Open file' button to browse and select the genotype specification file for the analysis. In this walkthroughs we are uploading the *'genotype specification file sleep.xlsx'* which is stored in the Example folder.

1**. Project Name**: Users can assign a project name for the current analysis. Here we give a project name as 'project_example-1'

2**. Light on time (Clock time, 24hr format)**: This is the light onset time of the experiment. Here we entering the default time 8

3**. Light off time (Clock time, 24hr format)**: This is the light offset time of the experiment. We are entering the default time 20

4. **Lifespan (Yes/No)**: Users can select to perform lifespan analysis by checking Yes.

    1. **Activity threshold**: This is the minimum value of the activity to determine the lifespan of a fly. Enter the default value 2

    2. **Maximum days of lifespan**: Enter the default value 11 here. If the lifespan exceeds 11, *SleepMat* automatically assign lifespan as 11.

5. **Individual output file (Yes/No) :** SleepMat analysis produces different sleep and circadian parameters, which are stored in the excel files. By default, program do not produce separate files for each parameter, instead clubbed together in single excel files for each analysis. If the user wishes to generate separate files for sleep and circadian parameter, check Yes. In this example select the default No.

Sleep analysis

1. **Sleep analysis (Yes/No)**: Users can select to perform sleep analysis by checking Yes.

2. **Start day of sleep analysis**: Enter the starting day of sleep analysis. Here we enter the default value 1

3. **The number of days of sleep analysis**: Enter the number of days to analyze. We are entering the default value 4.

4. **Sleep analysis/Interval (No/ Yes)**: select Yes

5. **Per interval (hr)**: Enter a value 1

6. **Sleep deprivation (Yes/No)**: Sleep deprivation analysis can be performed by selecting Yes.

7. **SD day**: The day when the flies are sleep deprived is entered here. Enter a value 4

8. **SD starting time (Clock time, 24 hr format)**: Enter the value 2

9. **SD duration**: Enter a value 6.

10. **Minimum SD% threshold (Yes/No)**: Select No.

11. **Minimum SD%**: If you select No in the previous step, then this will be deactivated.

12. **Triage condition for sleep analysis:** Select the first condition, Last day of activity < 5

Circadian Analysis

1. **Circadian analysis (Yes/No)**: Select Yes to perform circadian analysis.

2. **Anticipation analysis (Yes/No)**: Select Yes to perform anticipation analysis.

3. **Eduction analysis (Yes/No)**: Yes

4. **Start day of eduction/anticipation**: Enter the starting day of anticipation/eduction analysis. Enter the default value 1.

5. **Number of days of eduction/anticipation analysis**: Enter the default value 4

6. **Traige condition for sleep eduction analysis**: Select the default condition *Last day of activity < 5*

Periodogram

1. **Periodogram (Yes/No)**: Select Yes to perform chi-square periodogram analysis for individual flies.

2. **Periodogram figure (Yes/No)**: If the user would like to produce an average periodogram figure for each genotype, then select Yes.

3. **Start day of periodogram**: Enter the value 1.

4. **Number of days of periodogram**: Enter the number of days of periodogram analysis as 4.

5. **Significance level**: Enter the default value 0.01.

6. *Traige condition for periodogram*: Select the default condition *Last day of activity <5*

Actogram

1. **Actogram (Yes/No)**: To produce double-plotted actogram figures for individual flies, select Yes.

2. **Start day of actogram**: Enter the default is 1.

3. **Number of days of actogram:** Enter default value 11.

After entering all values, click on the **Start** green button on the general input panel. Analysis will start and the status will display on the **Status update** window. Now GUI should like this

If everything goes well, at the end you will get a message "Analysis completed!" on the Status Update window. All the results will be stored in a folder project_Example-1 (or the project name that you give). The folder should like this