SleepAnal2 is a Matlab application aimed to produce sleepograms from BigBrother data for individual bees and for the means ± s.e.m of user defined treatment groups. The program also outputs raw sleep data in an xlsx output file.

1. **Prepare input files:**

You will need to prepare two input .xlsx files to feed in to the application. The first is the file containing data from BigBrother. The second contains the grouping and filtering information for analyzing the data file.

1. Prepare the input Data .xlsx file:

* Open .bbdd obtained from the BigBrother using BigBrother viewer
* Export the file to excel. You will receive a .csv file in the same folder.
* Open the .csv with Excel and save it as .xlsx. eqach column in the file represents a BigBrother cell, including the cell name (1A1, 1A2 etc.)

1. Prepare the Group and Filter .xlsx file:

* Open a new xlsx file.
* In one column (Group column), give a treatment label using serial numbers (not zeros) to each BigBrother cell
* In one column (Filter column), give either 1 (filter) or 0 (include) value to each BigBrother cell
* **Important: do not leave empty cells in the Group and Filter columns**

1. **Analyze:**
2. In Matlab prompt, type: SlepAnal2 and press Enter. A dialog box will open.
3. Enter the path to the folder containing your Data and Filter and Group .xlsx files
4. Enter the Data .xlsx file name, including extension
5. Enter the range of the data that you wish to analyze in the Data . xlsx file, **including the header row!**
6. Enter the Group and Filter .xlsx file name, including extension
7. Enter the range of the Group and Filter columns. **Important:** the columns’ length must match the range of BigBrother cells that you defined in **d.**
8. Enter the time of the beginning of BigBrother data recording (zero time), found in the Data .xlsx file in cell A1. **Follow the specified format for date and time**
9. Enter the analysis start time. Must be equal or later than the zero time.
10. Enter the analysis end time. Must be later than the start time and erlier than the end of recording.
11. Enter the width of the window used to smooth the sleep plots in minutes
12. Enter the minimal duration of inactivity defined as sleep (default is 5 minutes)
13. Note whether you want to display error bars in the plots, or just means for each treatment group
14. Enter the name output .xlsx file
15. Note if you want to plot individual plots for each bee.

After program run, an xlsx output file.pdf figures are generated.

**Important: when running a new analysis, the generated new files will overwrite the files from previous analyses.**