How to calculate *C. elegans* sleep?

Download all the files from GitHub. Group all files but TenChamberSingleFileV1.py into a folder (named WormGUI\_10 here as an example) and save it to the Matlab folder.

**I Image analysis**

1. Open Matlab. Open the WormGUI\_10 folder from the “Current Folder” window in Matlab interface.
2. Open WormGUI\_new.m file in Matlab. Click the green color **Run** button above the Matlab editor window. A pop up window should appear.
3. The WormGUI popup window needs to know where your image files are stored on the hard drive. Copy the path name

(*e.g.* C:\Users\MBLUser\Doculments\MATLAB\A5JD1ND) for the folder containing your image files and paste it in the appropriate place in the WormGUI popup window. Add the name of your first image file to the path name

(*e.g.* C:\Users\MBLUser\Doculments\MATLAB\A5JD1ND\A5JD1ND\_t0000**.jpg**)

*Double check that you have the complete & correct file name and path.*

1. Do the same for the path and name of your last image file.
2. Select a Chamber number using the buttons to the left. Click “Mark region” to open the first image. Draw a box around the designated chamber. If using 6 chamber chips, ignore number 7 through 10. But you will have to mark 6 chambers, even if you are uninterested in empty chambers, 2 worm chambers, etc. The boxes you draw here do not need to be equal in size. If using 10 chambers, you will have to mark all 10 chambers. It is possible to mark less number of chambers, but you have to make a few changes in the Python script for data analysis accordingly.
   * 1. Note: you can and should exclude from your “box” areas of the chamber where air bubbles appeared—if the animal never entered that space during the entire movie.
     2. Note: if air bubbles disappear/appear in your movie in the initial or final frames of your movie, you may be able to exclude those frames from your analysis at a later step.
     3. Note: For your blank chamber, you can exclude areas where air bubbles appeared/disappeared.
3. Click the “Run” button in the WormGUI pop up window to start the analysis. Error codes may arise because of how Zeiss handles jpeg images. If WormGUI hangs up, restart WormGUI.
4. Save your results using same file name.txt (*i.e.*A5JD1ND**.txt** ). The default saving to the Matlab folder is preferred.

**II Quiescence calculation**

Make sure you have Python installed on your computer. To run the Python script for the analysis, the following should be installed (All the programs should be freely available):  
Python 2.7.3 or up  
Numpy 1.6.2 or up  
Scipy-0.10.1 or up  
Matplotlib-1.1.1 or up

Before analyzing the ‘filename.txt’ you just got from running ‘WormGUI\_new.m’, a few terms have to be defined first.   
1) Lethargus entry was defined as fractional Quiescence (fQ) stays above 0.1 for at least 20min. Lethargus entry was further adjusted to be the first frame of the first bout from the 60 frames used to calculate fQ.  
2) Likewise, lethargus exit was defined as fQ stays below 0.1 for at least 20min. Lethargus exit was further adjusted to be the last frame of the last bout from the 60 frames used to calculate fQ.  
3) The entire lethargus was divided into early, middle, late, overrun1, and overrun2 (E, M, L, O1, O2), each for roughly 45min. In order not to disrupt bouts, all bouts spanning two stages are included into both stages. Therefore, summary of total # of bouts from all five stages will not necessarily be equal to # of bouts for the entire lethargus.  
4) For each stage, total sleep (min), average bout duration(s), total # of bouts and bout frequency(/h) is calculated. Note: total sleep within stages is calculated without applying the 0.1 fractionalQ threshold. This means animals can have Q outside the length of lethargus. Q episodes that span two stages are not split, rather the stage is expanded to include episodes spanning both edges.

To run the analysis, right click ‘TenChamberSingleFileV1.py’, select **Edit with IDLE**. The Python script will be opened. Find the line with path=r’C:\Users\...\...\...txt’. Substitute ‘C:\ Users\...\...\...txt’ with the path for your file. Make sure you do not accidentally delete any part of the quotation mark (‘’) and the ‘r’ in front of the quotation mark. Choose **File**, click **Save** (or Ctrl+s). Choose **Run**, click **Run Module** (or **F5**). A Python Shell will popup. Once you see two ‘>>>’, the analysis is done. Now, in the same path as your .txt file, there will be two extra files. One shows you the fractional quiescent over frames and the other contains the detailed analysis for each chamber.

If you didn’t select all 10 chambers when running WormGUI, please make the following changes in the Python script. After the path definition, you will see “ChamberN=10”. Please change 10 to your number and follow the above instructions. I haven’t debugged all the numbers within 10, but 8 definitely works.

Extra features: The Python script takes the curve with the biggest area underneath as the real lethargus curve. Sometimes an adult animal would be too big for the chamber and got stuck, which will create a big curve before the movie stops. To ask the program to calculate the real lethargus, you can make it ignore certain frames. To do this, scroll down the Python script to about 2/3 of all lines. Find “#wormstatus=[item[:3500} for item in wormstatus]”. Delete the “#” and substitute 3500 with a number best for your analysis. This way, the program will only analyze the first 3500 frames and not the following ones. Once done with the analysis, please add back the “#” and save the script.

Declaration: The instructions here are for programs run in windows. If run in Mac, please make appropriate modifications.