Version: MC_QuiescenceActivity_v1202

WorMotel Analysis GUI ReadMe

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1. Click "Guide" in the MATLAB command window and open the GUI using the "Browse" tab.

2. If images are already processed, click the "Quiescence Analysis" tab. Otherwise, first process the images. Images should be processed using the GUI under the "Process Images" tab (see Image Processing ReadMe).

3. Click "Load Processed Data". Choose the file containing the processed image data.

4. Click "Set Parameters". Choose the start and end frame, quiescence threshold, and moving window over which to calculate the fraction of quiescence. Make sure "End Frame" is less than or equal to the total number of processed frames. The default quiescence threshold is 1. Quiescence threshold denotes the number of pixels required to move to call a worm active between two frames. The default moving window duration is 600 seconds (10 minutes).

5. Click "Compute Fraction of Quiescence".

6. Click "Group Mutants". Here you will tell the program which wells correspond to which experimental condition. The GUI accepts either 1 or 2 experimental conditions. If you have more, you will need to run the GUI more than once.

For no grouping, choose 1 and press ok.

For 2 experimental conditions in which worms are arranged in a flag pattern, click 2.

(RECOMMENDED) For manually entering the wells that contain each condition, choose 3. In the MATLAB command window, enter the well numbers containing genotype 1. Places square brackets around the numbers. Example: If wells 1 through 12 contain genotype 1, type "[1:12]" or "[1 2 3 4 5 6 7 8 9 10 11 12]" into the MATLAB command window (without quotation marks). Repeat for genotype 2.

7. After completion of grouping mutants, you will immediately be asked to delete undesirable regions of interest from genotype 1 and (if applicable) genotype 2. In the GUI window, simply click the rows containing data in which fraction of quiescence peaks are not clearly visible. Wells in which fraction of quiescence peaks are not visible by eye will not yield high quality data. When finished choosing ROIs to delete, press enter. In the Matlab command window, confirm or deny the deletion of these wells by inputting 1 or 0, respectively. If applicable, repeat for genotype 2.

8. After deleting undesirable ROIs, you will be asked to save data. This is recommended not necessary if moving on to the alignment stage.

9. Alignment (*for measurement of quiescence during development*): Click "Manual Alignment". Click a row with clear fraction of quiescence peaks. Manual alignment is used to crop fraction of quiescence peaks other than the L3 and L4 lethargus peaks. This is necessary if some L2s were included in the analysis. On the plot, click the start time and end time you want to include in the analysis. Choose a start time before the L3 lethargus, and choose an end time after the L4 lethargus.

10. You will now choose the start time for all regions of interest. The end time is chosen based on the duration of the time window chosen in the example in step 9. Choose a start time before the L3 lethargus. This process results in a crude manual alignment of the fraction of quiescence peaks. Once finished, accept or deny the manual alignment by inputting 1 or 0, respectively, in the MATLAB command window. If applicable, repeat the process for genotype 2.

11. Click "Digital Alignment". Input 1 to save the data.

12. Click the "Plot Data" tab. In this step, you will quantify quiescence and export the data to a .csv or .mat file.

13. Click "Load Analyzed Data". Choose the data saved in step 11. Input 1 into the MATLAB command window.

14. Click "Quantify Quiescence". Choose the fraction of quiescence threshold, Quiescence threshold, and strain names. Quiescence is quantified in the following way: in a user specified time window, each frame during which 1) the activity is less than the quiescence threshold AND 2) the fraction of quiescence is greater than fraction of quiescence threshold are counted toward total quiescence.

15. Use the pointer to choose the time window during which to quantify quiescence for genotype 1. If quiescence during L4 lethargus is desired, choose a start time before L4 lethargus and an end time after L4 lethargus. If applicable, choose the start time for quantifying quiescence in genotype 2. The duration of the time window is assumed to be the same as for genotype 1.

16. Repeat the process of step 15 to choose a time window for activity measurements. This is done to measure the average activity of each strain. It is recommended to measure the average during the L4 stage.

17. Click "Save Data". Enter 1 for a .csv file (Excel compatible) or 2 for .mat file (MATLAB). Choose a save name for the file. The saved file will contain vectors containing various properties. The size of the vectors will equal the number of worms contained in the analysis. The average activity, total quiescence, L4duration, and well number are returned for each worm. Well number is used to track which experimental condition each worm came from.