

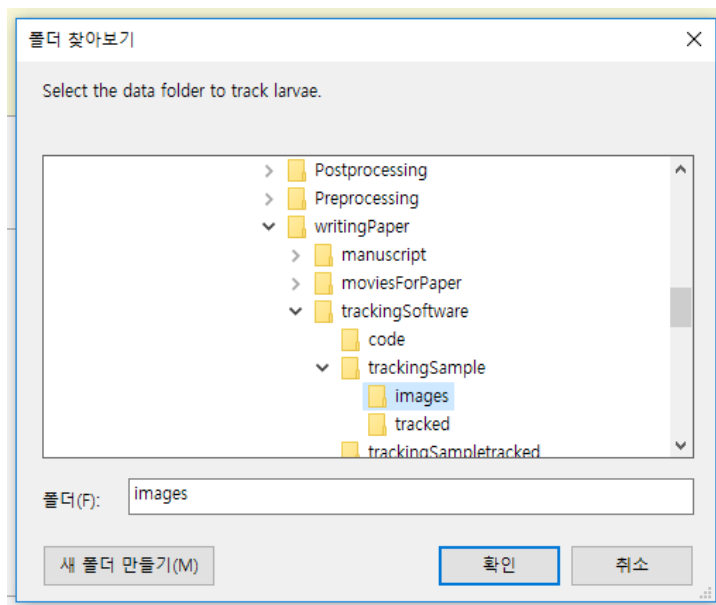
## User's guide for dig-and-dive tracking software

Matlab routines developed to analyze the behavioral movies of digging and diving larvae presented in the following eLife publication: *Species-specific modulation of food-search behavior by respiration and chemosensation in Drosophila larvae* (2017) by Kim, D., Alvarez, M., Lechuga, L., and Louis, M.

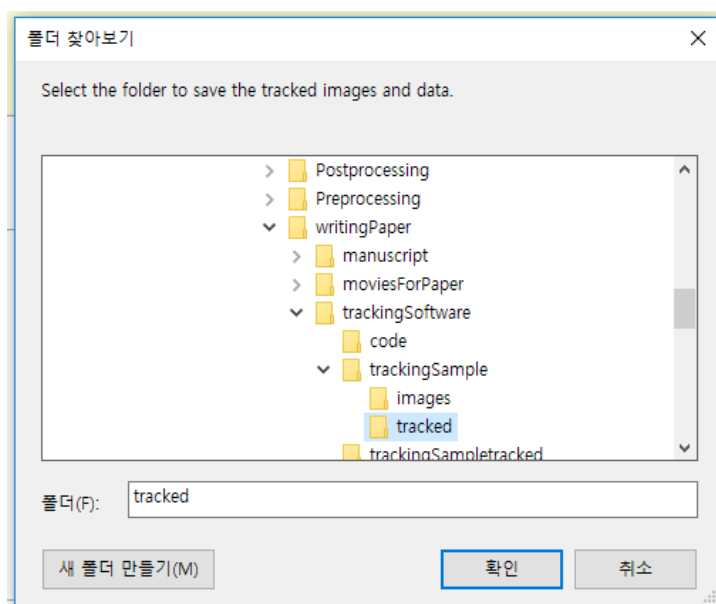
To run the scripts of the dig-and-dive tracking software, please follow the instructions below.

### For tracking larvae,

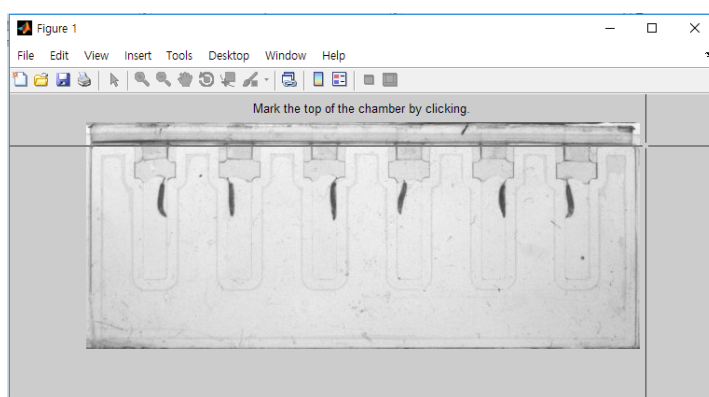
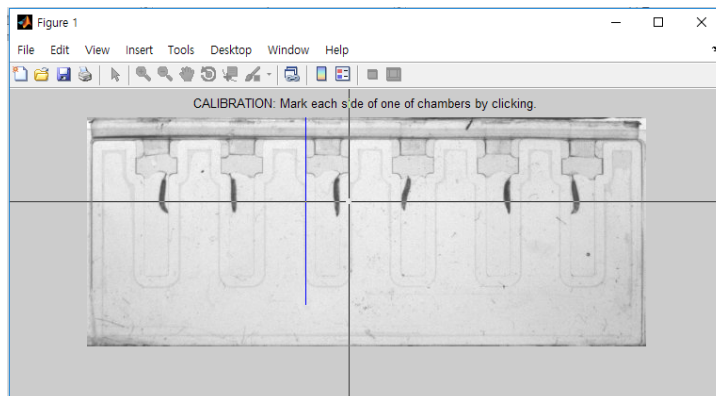
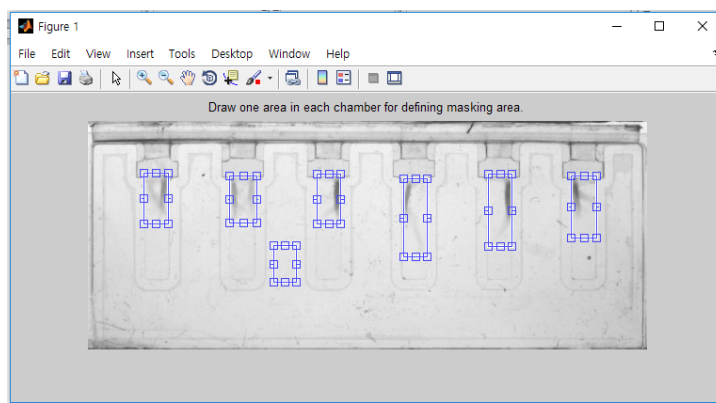
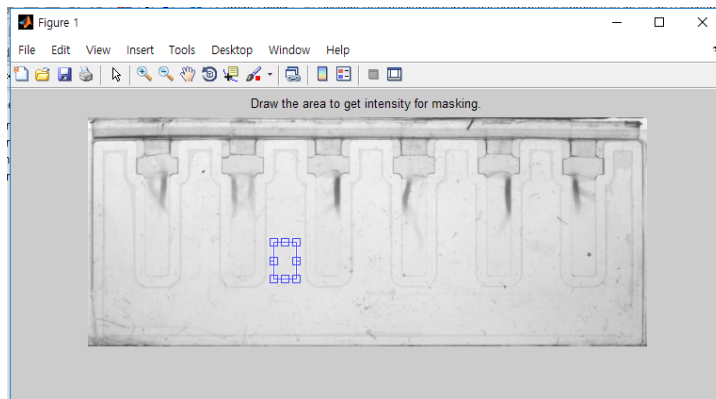
1. Run the script "DnDtracker.m" through the Editor GUI of Matlab.
2. A pop-up window will appear. Select the input folder containing image sequence to be used for the tracking. For the tutorial, select the folder "images" in the software package.

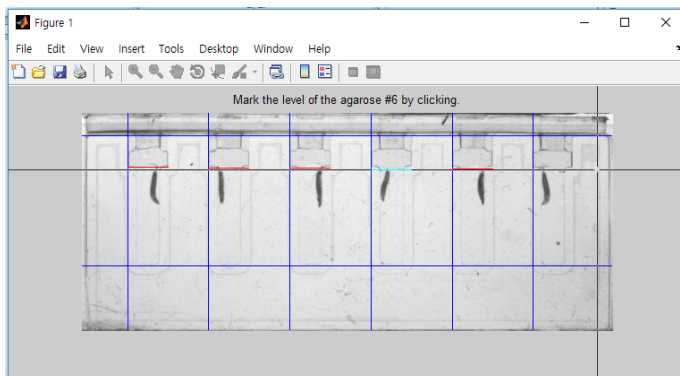
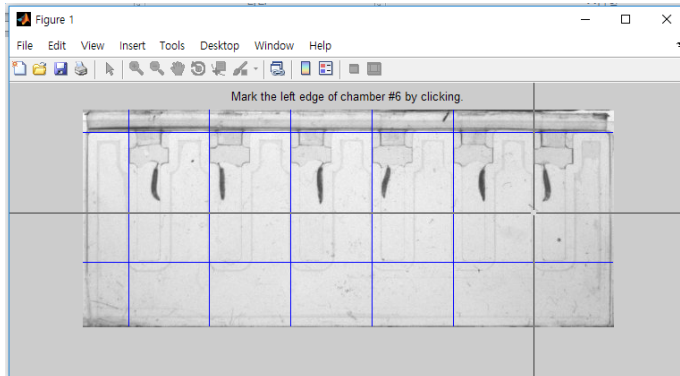
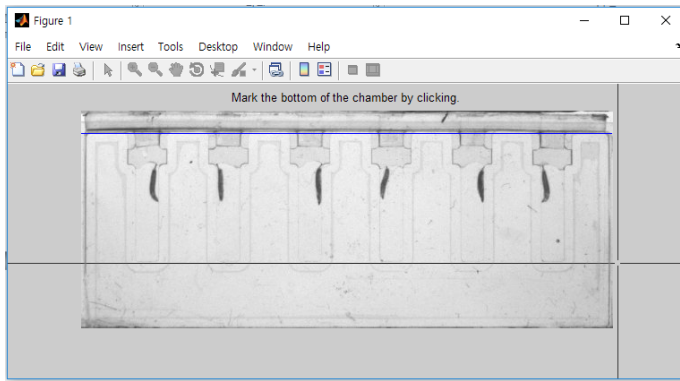


3. Then, another pop-up window will open. Choose an output folder where you want to store all output files generated by tracking and behavioral annotation programs. For the tutorial, select the folder "tracked" that is included in the software package.



4. After a while, several images will pop up one by one. Follow the instruction displayed at the top of each figure window as shown in the screen shots below. Mark the desired position on the screen with the crosshead:

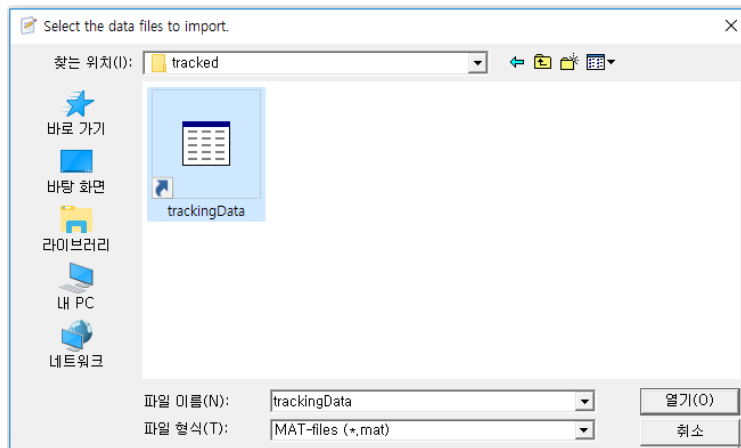




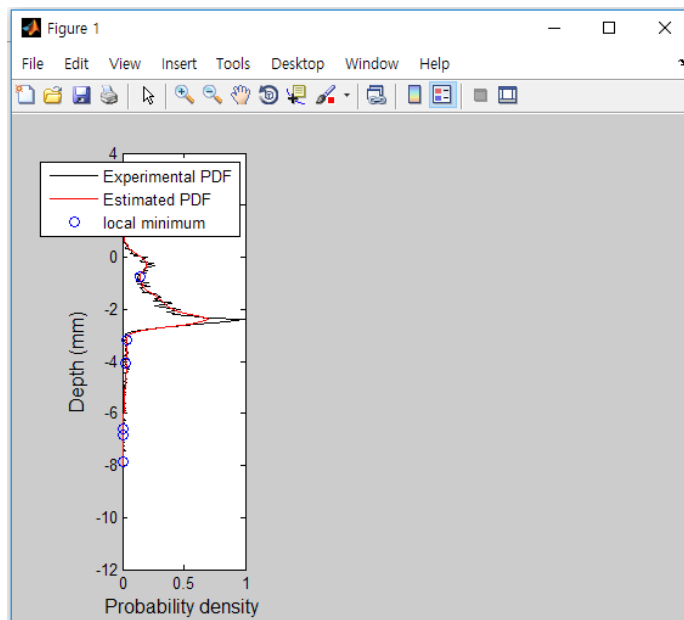
After completion of tracking, all output files will be stored in the output folder.

**To enter the threshold values based on which the behavioral modes are defined and classified, follow the next steps:**

1. Run the script, "thresholdAnnotation.m"
2. A pop-up window will open. Select the tracking data file in the output folder that you generated after the tracking. For tutorial, select "trackingData.mat" in "tracked" folder. If you have more than one data file (e.g. "trackingData1.mat, trackingData2.mat, etc"), you can select multiple files. After running this script, the results will be merged in a single output file "trackingDataAll.mat".



- Once the file has been selected, a figure window will appear in the screen displaying the probability density distribution of the vertical position of larval centroids. Follow the instruction printed in the command window while observing the figure to pick the most appropriate threshold value to define the “diving” mode. The other thresholds are pre-defined by default.



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Command Window
New to MATLAB? Watch this Video, see Demos, or read Getting Started.

dataFile =

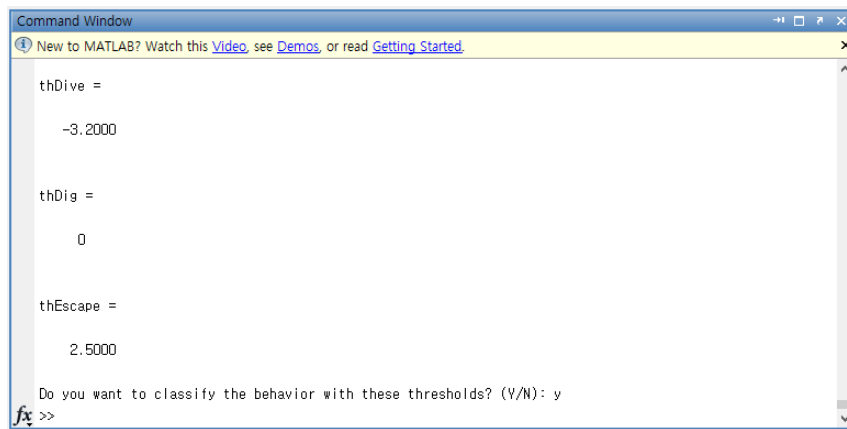
trackingData.mat

ans =

-7.8500 -6.8500 -6.6000 -4.0500 -3.2000 -0.7500

Diving threshold value is considered as location of the first minimum
below the head of the estimated PDF.
fx Enter the index of threshold for diving in the above list of values: 5
  
```

4. If you want to classify behaviors with thresholds that you define yourself, you can enter “y” in the command window. The annotation data will be attached to the final output file, “trackingDataAll.mat”.

A screenshot of the MATLAB Command Window. The window has a title bar that says "Command Window". Below the title bar is a yellow banner with a blue information icon and the text "New to MATLAB? Watch this [Video](#), see [Demos](#), or read [Getting Started](#)". The main area of the window is white and contains the following text: "thDive =", "-3.2000", "thDig =", "0", "thEscape =", "2.5000", "Do you want to classify the behavior with these thresholds? (Y/N): y", and "fx >>". The "fx" icon is a small blue square with a white 'f' and a red 'x'.

We hope this script will help you carry out successful analysis of your data. Do not hesitate to send us any comments to improve these scripts.

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