# DuoMouse instructions

The instructions for the DuoMouse software comprise five pages: "Setting", "Recording", "Tracking", "Analysis", and "View".

Setting

This page allows you to enter the basic settings for the video analysis. You cannot start capturing or analyzing data without entering information here. After the basic information has been entered, the four other buttons ("Start recording", "Start tracking", "Start analysis", and "View chart") will appear.
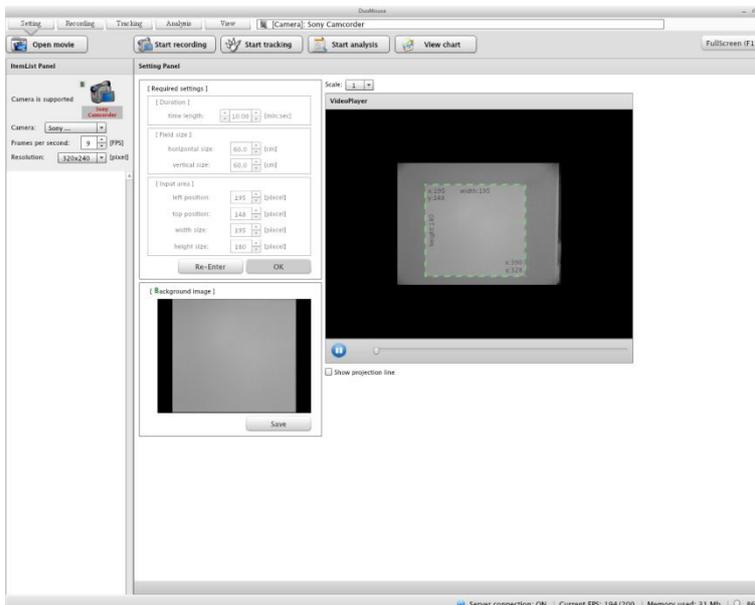

1. Press the camera icon ( ) to show the real-time image from the camera. *If you want to analyze an already existing video file (FLV file), click "open movie" and select the folder that contains the video file. Then double click the file from the Item List Panel (left side). ** You can change the resolution of the video file, but a high resolution may slow down the speed of video recording depending on the spec of your computer. We recommend that you use the default setting (Resolution: 320×240, Frames per sec: 6 frames/sec).

2. Enter the duration of the test (min) in the panel at the top right corner.

3. Enter the size of the open-field (horizontal and vertical size, cm).

4. Select the area (of the bottom surface of the open-field) by adjusting the size of green dotted line square and then press "OK". This area should correspond to the size of the open-field that you entered at step 2.

5. Press "Save" to capture the background image of the test field **without any mice present**. This background image will be saved as "###_bg.png" file.

6. DuoMouse uses this image as the background image for all the video capture, so you do not need to set the background for each session. If you want to change the test situation e.g. change the size of field or light intensity, please change the parameters or retake background image using the Setting page (step 2) before recording the new test.

Recording

This page enables you to record real-time behavior into an FLV video file for further analysis.
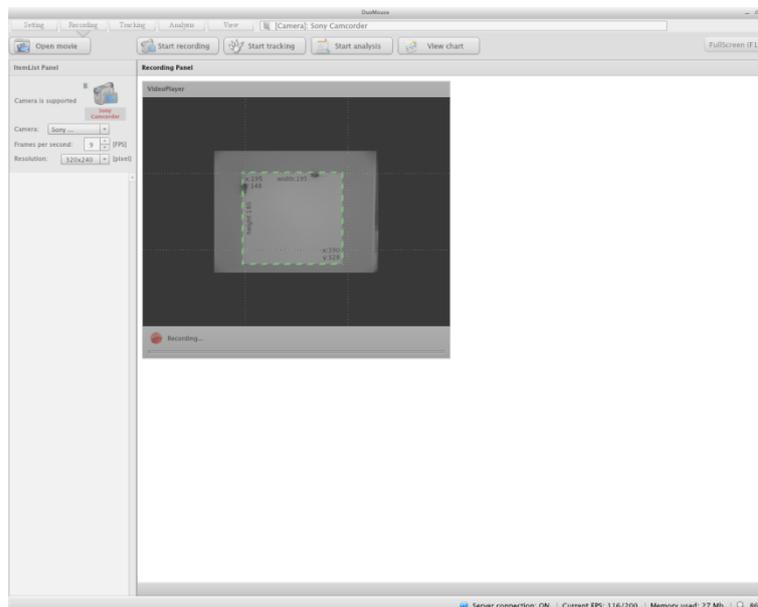
1. Introduce two mice into the open-field.
2. Click "Start recording".
3. DuoMouse records the video and automatically stops the video capture process after the decided duration (depending on the setting that you entered initially).
4. After the session has been completed, you will be asked to save the session file.

Tracking

This page enables the positions and movements of two animals in the field to be tracked.
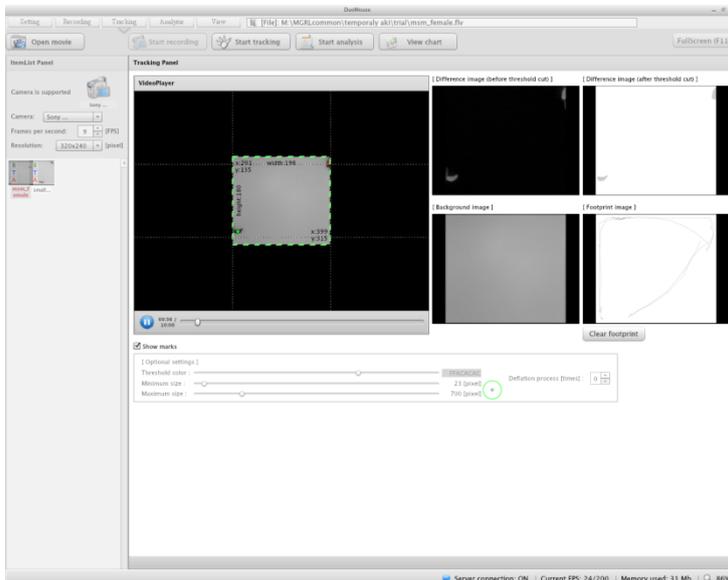
1. Click "Open movie".
2. Select the folder that you want to analyze. (Steps 1 and 2 can be skipped if you want to track files that have just been recorded.)
3. Before starting the tracking, please enter the following settings. Please open one video file by double clicking the file in the Item List Panel when you decide these settings.

   [Threshold]   Detection criteria for the contrast between the background and the image. If the contrast between your mice and the test-field is small, you will need to adjust the threshold to track the mice properly.

   [Minimum size]   Detection criteria for the minimal particle size of an animal (pixels).

   [Maximum size]   Detection criteria for the maximal particle size of an animal (pixels).

   * You will see a green double circle under these settings. The outer circle corresponds to the pixel size of the selected maximum size, and the inner circle corresponds to the minimum size. You can drag these circles to move them if you want to compare the pixel size and the difference image of the mice (in the four panels on the right).

4. Select the files that you want to track from the Item List Panel. You can select several files by selecting them with the left-hand mouse button.

5. Press "Start tracking".

6. The results of the tracking will be saved automatically in the same folder as the movies. The tracking results will be in four files: "xy position data (###_xy.csv)", "Four observable parameters (###_obs.csv)", "Histogram bins of four observable parameters (###_obsH.csv)" and "tracking image (###_track.png)". ### corresponds to each file name.

xy position data (_xy.csv): gives the positions of the two mice in each time frame. DuoMouse detects the positions every 0.33 sec (3 frames per second). If your test session is 10 min, you will obtain 1800 data points (rows). The results file contains five columns: 1st column, frame number; 2nd column, x-axis position (pixels) of animal #1; 3rd column, y-axis position (pixels) of animal #1; 4th and 5th columns, the x- and y-axis positions of animal #2, respectively.

Four observable parameters (_obs.csv): Results for the four observable parameters that will be used for the HMM estimation. (a) The distance between two mice (1st column), (b) the relative angle of the velocities of two mice (2nd column), (c) the relative speed of two mice (3rd column), and (d) the average speed of two mice (4th column).

Histogram bins of four observable parameters (*###_obsH.csv*): Transformed values of four observable parameters into ten bins of histograms. This values are used for the HMM estimation.

Tracking image (_track.png): Tracking image of the two mice.

*As an option, you can also change some detection criteria, including motion life and noise. *Motion life* can be used to fill in gaps between frames. When DuoMouse fails to detect a mouse in some frames, the previous position of that animal will be retained as the current position to fill the gap. The duration set for motion life determines the duration of retention of the previous position. *Noise_size [pixel]* is used to smooth the edge of the difference image.


Analysis

This page enables the behavioral state to be analyzed by using the HMM (both 2-state and 3-state HMM). To perform this analysis, DuoMouse requires internet access. DuoMouse sends the tracking results via the internet to our server (MGRL, NIG, JAPAN) to calculate the HMM estimates and then returns the results to your computer.
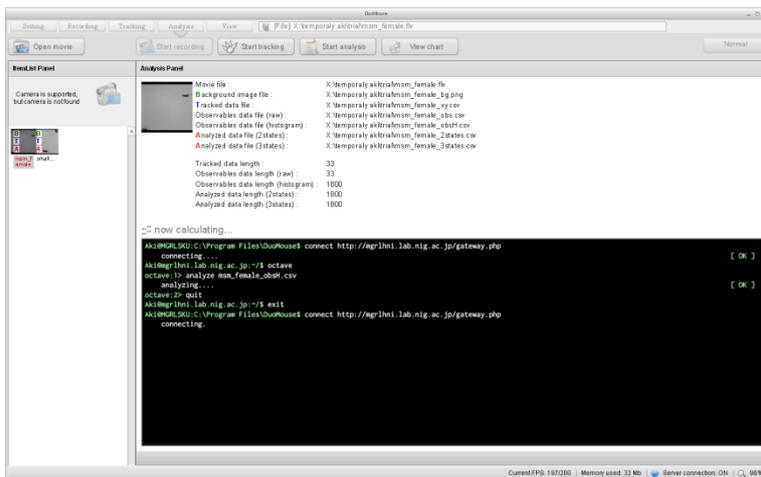
1. Select the files that you want to track from the Item List Panel. You can analyze several files together by selecting them with the left-hand mouse button.
2. Click "Start analysis".
3. It will take a while to return the results (about 60 sec per file for a 10-min test).
4. When all the analyses are finished, you will see "Complete" pop up.
5. The result of the analysis will be saved automatically in the same folder as the movies. The tracking results will be in two files: "2-state HMM (###_2states.csv)" and "3-state HMM" (###_3states.csv)". ### corresponds to each file name. For the 2-state HMM, "0"="indifference" and "1"="interaction". For the 3-state HMM, "0"="indifference", "1"="sniffing", "2"="following".

View

This page enables you to view the results for the four observable parameters and the behavioral states that were obtained by the HMM analysis.
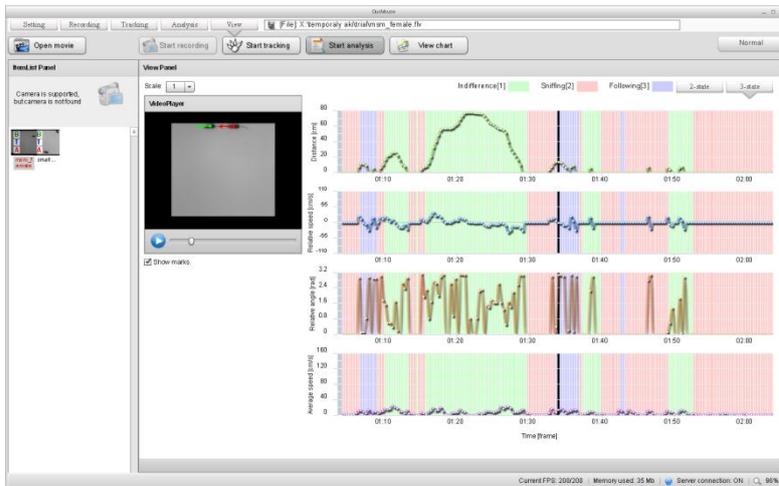
1. Select a file for which you want to view the result from the Item List Panel.
2. Click "View chart".
3. Select either "2-state" or "3-state" hidden Markov model (top right).
4. Play the video by clicking play (). You will see the result of the behavioral states as the background color of the figures (For the two-state HMM, green: indifference, and pink: interaction. For the three-state HMM, green: indifference, pink: sniffing, and purple: following). The horizontal black bar traces the current state of the mice in the figure for each of the four observable parameters.