

Instructions – GUI and Analysis

- **GUI DESCRIPTION**

Load Video = Load/reload video file for grooming analysis. The currently supported file formats are: .AVI, .MOV, .MPEG, .MP4, .SEQ, .TIFF

Save Path = Set the path to the directory where you want to save the output file. Users can select any arbitrary name for the output file, to be saved as .TXT, .XML, .CSV or .HTML. M-Track exits and closes the GUI if no directory is selected

Start CAM = Start Continuously Adaptive Meanshift algorithm

Exit = Close M-Track GUI

Draw Cage = Draw the perimeter of the bounding box in which you want M-track to perform the tracking analysis

Draw LF ROIs = Draw the perimeter of the box representing the region of interest (ROI) that M-Track track uses to identify the color of the left foot. It is important to draw this ROI *inside* the area of the colored forepaw. To draw the left foot ROI, click the left mouse button on the top left corner or the ROI, drag the mouse and release the left mouse button from the bottom right corner of the ROI.

Draw RF ROIs = Draw the perimeter of the box representing the region of interest (ROI) that M-Track track uses to identify the color of the right foot. It is important to draw this ROI *inside* the area of the colored forepaw. To draw the right foot ROI, click the left mouse button on the top left corner or the ROI, drag the mouse and release the left mouse button from the bottom right corner of the ROI.

Detect Mice = Visualize the mouse body ROI

Detect LF = Visualize the left foot ROI

Detect RF = Visualize the right foot ROI

Mice = Number of mice to be tracked (available options: 1, 2, 4)

Execute = Start the analysis

Pause = Pause the analysis

Viewing Mode = View the video in its acquisition format (Original), in the HSV color space (HSV) or in three distinct mask modes that allow the user to set the lower and upper HSV limits to isolate the color of the body (Body Color Mask), left (Left Foot Color Mask) and right foot (Right Foot Color Mask)

L Hue = Set the lower limit for hue

L Sat = Set the lower limit for saturation

L Val = Set the lower limit for value

U Hue = Set the upper limit for hue

U Sat = Set the upper limit for saturation

U Val = Set the upper limit for value

Dilation = Enlarge the size of the body and feet color masks

Min Box Size = Minimum number of pixels contained in the mouse body, left and right foot ROI

Collision Detect = Selecting this option allows M-track to detect only one body color mask per mouse

Noise Reduction = Remove noise in the image by using a non-local means denoising algorithm, using the selected denoise value

Denoise Val = Regulate the strength of the noise reduction

Zoom = Zoom within the cage area

The color box in the GUI display the colors of the lower (left) and upper limits (right) for the selected HSV settings.

- **VIDEO ANALYSIS INSTRUCTIONS**

1. Press **Load Video** to upload the video file to be analyzed. M-Track analyzes 8-bit video files. The supported video file formats are: .AVI, .MOV, .MPEG, .MP4, .SEQ, .TIFF.
2. Press **Save Path** to select the directory where M-Track saves the output data. Users can select any arbitrary name for the output file, to be saved as .TXT, .XML, .CSV or .HTML.
3. Press **# Mice** to select the number of mice to be analyzed (1, 2, 4).
4. Press **Draw Cage** to draw the perimeter of the behavioral arena where M-Track performs the tracking analysis. M-Track expects the perimeter of the entire behavioral arena to be shaped as a box. This box is drawn with three consecutive click-and-release steps using the left mouse button. If the analysis is performed on more than one mouse (i.e. 2 or 4), M-Track allows the user to first draw the perimeter of the bounding box for the behavioral arena, as described above. Next, a pop-up message requests the user to **Now draw a line for each dividing wall**. Only one line needs to be drawn when analyzing two mice, whereas two lines need to be drawn when analyzing four mice. The lines are drawn by clicking and dragging the left mouse button.
5. In **Viewing Mode**, select the option **Body Color Mask** and set the lower (L) and upper (U) limits for the HSV settings to isolate the color of the mouse body from any other color present in the background of the image. The **Dilation** button allows to enlarge the area of the Body Color Mask. The **Min Box Size** can be used to set a lower threshold for the number of pixels contained within the area that M-Track identifies as the Body Color Mask ROI. This is useful to reduce tracking errors when other pixels, in the enclosure, have the same HSV settings as the fur. Skipping this step does not allow the user to draw the left/right foot ROIs for the tracking analysis of multiple mice.
6. Press **Draw LF ROIs** to draw a box representing the ROI that M-Track track uses to identify the color of the left foot. It is important to draw this ROI **inside** the area of the colored forepaw. To draw the left foot ROI, click the left mouse button on the top left corner of the ROI, drag the mouse and release the left mouse button from the bottom right corner of the ROI. When analyzing multiple mice, the user needs to draw the left foot ROI for the marked foot of each mouse.
7. Press **Draw RF ROI** to draw a box representing the ROI that M-Track track uses to identify the color of the right foot. It is important to draw this ROI **inside** the area of the colored forepaw. To draw the right foot ROI, click the left mouse button on the top left corner of the ROI, drag the mouse and release the left mouse button from the bottom right corner of the ROI. When analyzing multiple mice, the user needs to draw the right foot ROI for the marked foot of each mouse.
8. In **Viewing Mode**, select the option **LF Color Mask** and set the lower (L) and upper (U) limits for the HSV settings to isolate the left foot area. The **Dilation** button allows to enlarge the selected foot area. The **Min Box Size** can be used to set a lower threshold to the number of pixels within the left foot ROI. This is useful to reduce tracking errors when other pixels, in the enclosure, have the same HSV settings as the left foot.
9. In **Viewing Mode**, select the option **RF Color Mask** and set the lower (L) and upper (U) limits for the HSV settings to isolate the right foot area. The **Dilation** button allows to enlarge the selected foot area. The **Min Box Size** can be used to set a lower threshold to the number of pixels within the right foot ROI. This is useful to reduce tracking errors when other pixels, in the enclosure, have the same HSV settings as the right foot.
10. In **Viewing Mode**, select the option **Original**. Press **Detect Mouse**, **Detect LF**, **Detect RF** to check that all HSV settings are optimally tailored for accurate grooming analysis by tracking the position of the mouse body, left and right foot, respectively.
11. As a final step, press **Execute** to run the M-Track analysis and **Pause** to pause the M-track analysis and reset the detection parameters if needed.

12. The **Start CAM** option allows the user to initiate the Continuously Adaptive Meanshift algorithm, through which M-Track rotates and rescales the LF and RF ROIs to locate the position of the mice LFs and RFs.

13. Press **Exit** to close M-Track.

We include two sample videos and the corresponding output trajectories. The videos provide examples of grooming episodes recorded in white and black mice. Frames may be missed when one of the forepaws is hidden by the mouse body (e.g. the two forepaws are on top of each other or one of them is behind the mouse body or covered by the tongue of the mouse). When that happens, the marking dot goes to the top left corner of the mouse box. In this case, the x,y coordinates for the missed forepaw are set to 0 in the output file.

- **Optimized settings to track grooming of the black-fur mouse (C57BL/6) in "Sample_Video_Black_Mouse.MP4"**

Body Color Mask

L Hue = 0
L Sat = 74
L Val = 0
U Hue = 180
U Sat = 255
U Val = 94
Dilation = 1
Min Box Size = 600
Collision Detect = ON

LF Color Mask

L Hue = 42
L Sat = 74
L Val = 97
U Hue = 70
U Sat = 207
U Val = 202
Dilation = 6
Min Box Size = 15

RF Color Mask

L Hue = 123
L Sat = 178
L Val = 138
U Hue = 134
U Sat = 219
U Val = 255
Dilation = 6
Min Box Size = 15

- **Optimized settings to track grooming of the white-fur mouse (Swiss Webster) in "Sample_Video_White_Mouse.MP4"**

Body Color Mask

L Hue = 0
L Sat = 0
L Val = 113
U Hue = 180
U Sat = 255
U Val = 255
Dilation = 1
Min Box Size = 700
Collision Detect = ON

LF Color Mask

L Hue = 44
L Sat = 20
L Val = 87
U Hue = 97
U Sat = 255
U Val = 124
Dilation = 6
Min Box Size = 10

RF Color Mask

L Hue = 0
L Sat = 112
L Val = 110
U Hue = 180
U Sat = 255
U Val = 255
Dilation = 6
Min Box Size = 10