

MouseWalker manual



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The MouseWalker program allows the tracking and quantification of the walking behavior of a quadruped using the frustrated Total Internal Reflection (fTIR) optical technique. The program can automatically identify and track footprints (FP), body features and body orientation. In addition, it allows the user to manually edit the tracked video so that the tracking is complete and properly done. Finally, it can statistically analyze the walking behavior by generating a large set of graphs and output the results in an excel spreadsheet with the quantification of gait and other parameters.

MouseWalker can analyze movies saved as image sequences. Note that only the sequence files can be present in the analyzed directory. The program can read the following graphic file extension formats:

BMP — Windows Bitmap	JPEG — Joint Photographic Experts Group	PNG — Portable Network Graphics
CUR — Cursor File	JPEG 2000 — Joint Photographic Experts Group 2000	PPM — Portable Pixmap
GIF — Graphics Interchange Format	PBM — Portable Bitmap	RAS — Sun Raster
HDF4 — Hierarchical Data Format	PCX — Windows Paintbrush	TIFF — Tagged Image File Format
ICO — Icon File	PGM — Portable Graymap	XWD — X Window Dump

Auto tracking is based on cluster identification created on the brightness of an image within preset thresholds, as well as information from preceding frames.

I. Quick guide

From acquiring the videos until quantification of the walking behavior, the following steps must be taken:

1. Set and verify the tracking settings. Importantly, scale calibration, threshold parameters and size features.
2. Complete auto tracking.
3. Cure the tracked video.
4. Statistically evaluate the tracked video.

Below, **Sections II** and **III** describe the program's main window and setup window, respectively. **Sections IV** describes the list of shortcuts; **Section V** discusses how to correct a tracked video; and **Section VI** includes troubleshooting and tips.

II. MouseWalker main window

MouseWalker can be opened either by running the executable file for the standalone Windows version, or running the “mousewalker.m” file in MATLAB. Once opened, the main window should appear (**Figure 1.**).

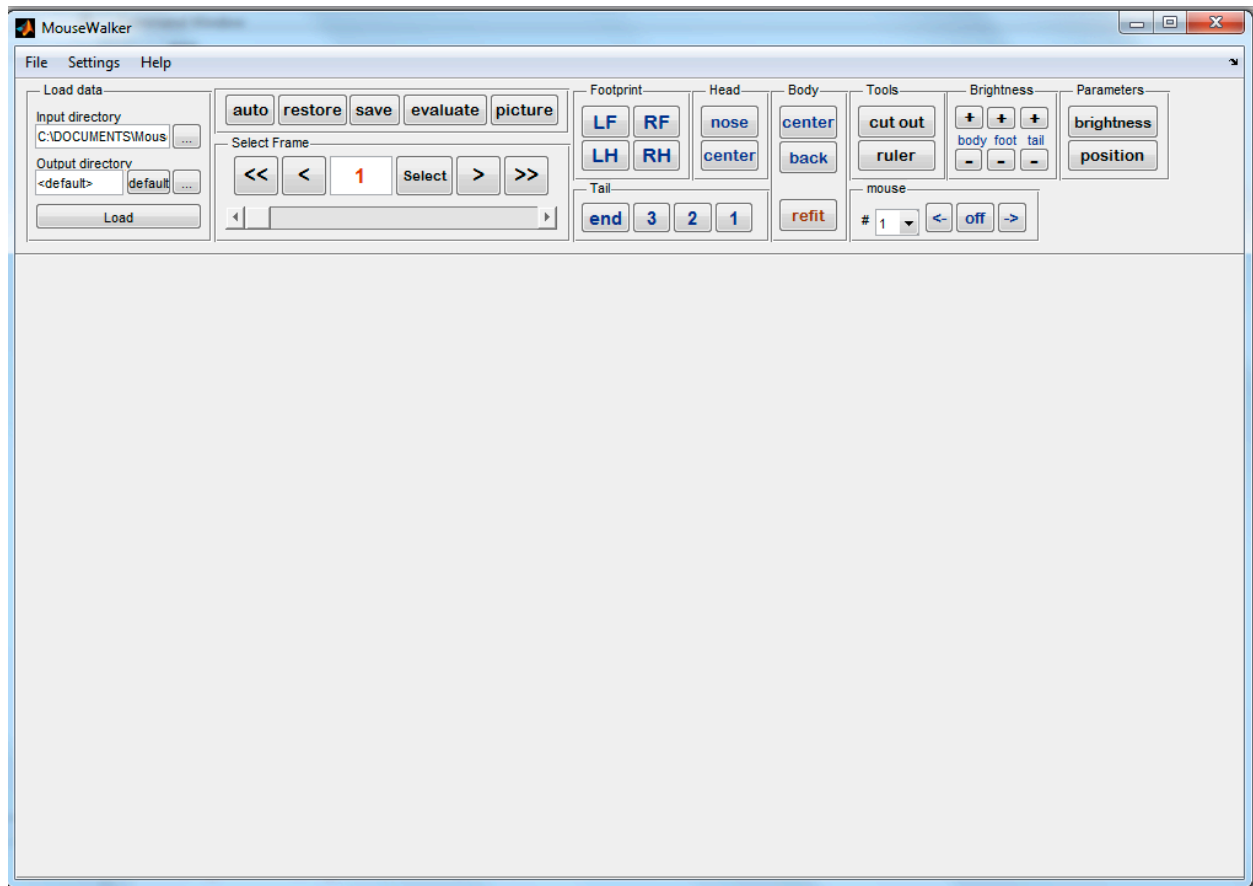


Figure 1. MouseWalker interface.

The main window is divided into several panels that allow the user to load the movies; scroll the movies; enable auto-tracking; cure the tracking and evaluate the tracked movie.

1. **Load Data:** this panel defines the directory where movie frames are loaded from (**Input Directory**), and the directory where results are saved to (**Output Directory**). The user can specify the output directory as “<default>”, either by typing this in or by clicking the

default button, which will be "<Input Directory>/Results/". Both directories can be typed in or selected from a browse menu, which can be accessed using the respective "...". Data is loaded by pressing the **Load** button. Note that Load also loads the output that was generated previously for the given video.

2. **Footprint:** for a particular frame, each leg can be turned on or off manually (or of course, could be turned on/off by auto-tracking). The legs that are present on the given frame shown on the screen are indicated by the pressed state of the respective buttons (L, R, H and F correspond to left, right, hind and fore, respectively). If a leg track is not currently present and the user decides to place it, they can press the respective leg button, after which they can place the footprint on the screen by clicking on the screen to the location where the footprint track should be placed. In this case, the footprint will be placed for the current frame as well as the consecutive frames to the same location, constantly until two frames before the next appearance of the same footprint. For example, if the right foreleg (RF) does not appear on frames #40-49, but appears on frame #50, placing the footprint on frame #40 will place it on frames #40-48. Removal will also be done for the consecutive frames following the present frame in which the footprint is present. If the button was pressed erroneously and the user is prompted to place the footprint using the mouse, clicking outside the screen will cancel the action.

3. **Body**

- a. **Center:** lets the user place the animal's body center manually using the cursor. After placing the center's location, a second point needs to be selected, which determines the direction of the body at the center, such that the direction points from the central point to the point that is selected second.
- b. **Back:** lets the user set manually the location of the back point of the animal, along with its direction (see Center).
- c. **Refit:** This button uses the currently selected location of the nose and the end of the tail, along with the body contour, to determine the rest of the body points.

4. **Head**

- a. **Nose:** lets the user set manually the location of the nose of the animal.
- b. **Center:** lets the user manually select the location of the center and orientation of the head.

5. Tail

- a. **End:** lets the user manually select the end point of the tail.
- b. **3,2,1:** lets the user manually select three points of the tail that are meant to be equidistant along the tail, along with the orientation of the tail at these points. 3 is the one closest to the end of the tail, while 1 is the closest to the body.

6. Tools

- a. **Cut out:** lets the user make a (rectangle) part of the image considered background for all frames. After pressing this button, the user can select two points on the screen, which will be two diagonal corners of a rectangle. All pixels within this rectangle will be considered background points for the analysis for all frames. If a mistake was made, where for example, the region where a footprint will or was located was removed, the user can press **Restore** (see below). This feature can be used as many times as necessary.
- b. **Ruler:** lets the user measure the distance in the image between two points. After pressing this button, the user can select two consecutive points. The distance between these two points will appear on the upper left corner of the image in both pixels and millimeters, for the latter using the mm->pix conversion specified in Settings.

7. Mouse

- a. **#:** lets the user select a mouse. The body part track selections described above will act on the selected mouse only. At a given frame, the currently tracked mice will be shown with plain numbers –such as “1.” Mice that are tracked but not on the current frame will be shown in parentheses –such as “(1).” If all tracks of a given mouse are deleted, the mouse’s number will change to “-” in the list and the mouse will no longer be trackable.
- b. **off:** turns off labeled footprints and body features of the selected mouse in the present frame.

- c. **->**: turns off labeled footprints and body features from the selected frame onwards. Useful to trim the tracking at the end of the video.
- d. **<-**: turns off labeled footprints and body features from the selected frame backwards. Useful to trim the tracking at the beginning of the video.

8. Brightness

- a. **+/-**: Increases (+) or decreases (-) the brightness of the body, tail or footprints as identified by the program, respectively, as seen on the screen (i.e. it doesn't affect the tracking that uses the brightness thresholds set in settings!). Useful to more easily identify the footprint onset/offset by the eye.

9. Parameters

- a. **brightness**: lets the user select a pixel in the image; the RGB brightness of the given pixel in the image will be shown at the upper left corner of the image (on a 0-255 scale for each color).
 - b. **Position**: lets the user select a pixel in the image; the x-y coordinates in pixel units starting from the upper left corner of the image will be shown at the upper left corner of the image.
10. **Select Frame / <N> Frames**: allows the user to scroll the frames of the movie. The total number of frames present is indicated in the place of <N>.
- a. **<<**: jumps to the first frame.
 - b. **<**: moves one frame backwards.
 - c. **Current frame**: the frame that is currently being visualized is indicated. Alternatively, the user can input the frame number manually and press **Select**.
 - d. **>**: moves one frame forward.
 - e. **>>**: jumps to the last frame.

- f. There is also a slider that can be used to choose frames.
11. **Auto:** once pressed, the program starts the auto-tracking of the frames starting from the current frame. This latter feature is important as the auto-tracking algorithm uses the leg positions on the previous frame for each frame for footprint-identification. For instance the user can specify the footprint locations on a given frame, and by starting auto-tracking on the next frame, the program will use the specified locations as initial condition.
 12. **Restore:** restores tracking information to the last saved status. I.e. restores footprint and body information, cut-out regions, etc.
 13. **Save:** saves all tracking information, as well as cut-out regions.
 14. **Evaluate:** statistically evaluates the collected tracking information, and creates a set of graphs and an excel spreadsheet. Quantification will only take into account tracked information (not untracked video). Note that the target file cannot be opened while the evaluation is ongoing, in case the same video is reevaluated. The files will be saved in a subdirectory where the original files were located (default directory: "Results"; thereafter Results folder). Incorrectly tracked videos will result in incorrect gait values.
 15. **Picture:** creates a subfolder within the Results folder, called "Images", and starts saving images of the frames of the input video, with the addition of tracking information, as seen on the screen. Image saving starts on the current frame, and goes on continuously until the user presses the Picture! button again, that terminates the process. To save the complete video, the Picture! button has to be pressed on the first frame of the video. The features that are saved on the sequence files can be set on the Setup window (see below).

III. Setup window

Before loading a movie (or starting the auto tracking), the settings must be properly set so that auto tracking and the measuring features are accurate. In order to open the Settings window go to Edit>Settings or press Control+S. Figure 2. displays the settings window.

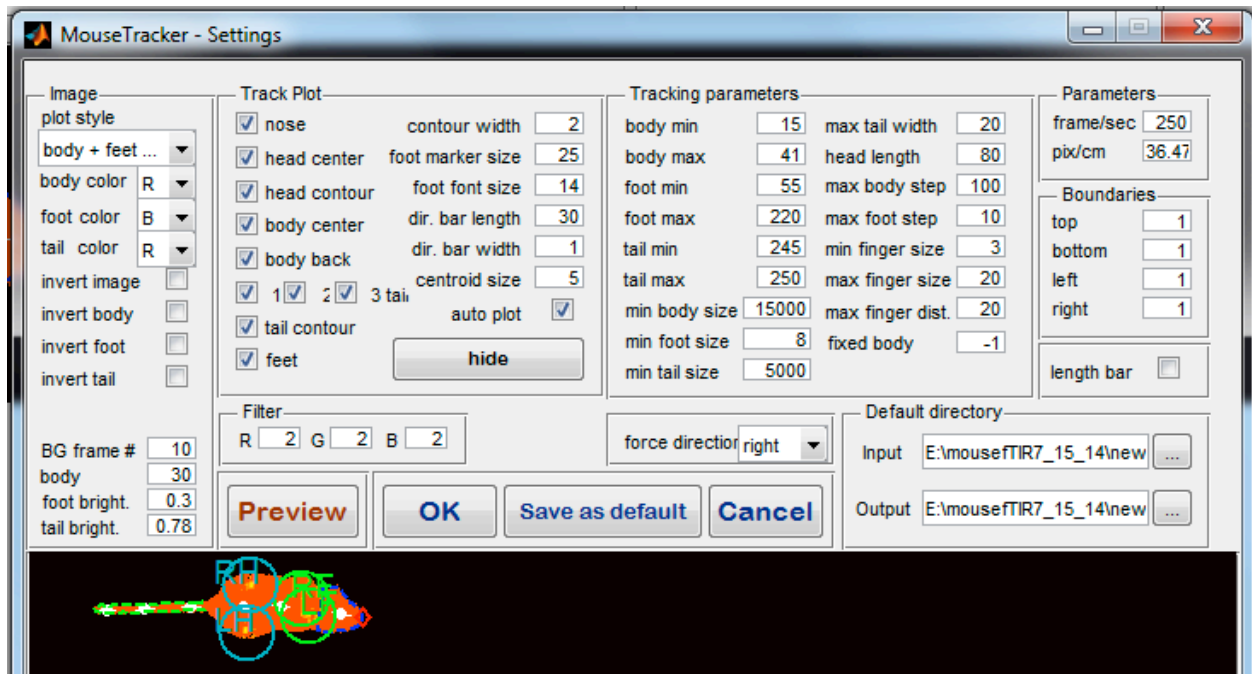


Figure 2. Settings window.

The Settings window is divided into several areas in addition to a preview button that display the effect of changing some of the settings. Settings can be set for a particular movie or set as default. For some features, particularly for the threshold values, the effects can be immediately observed by pressing the Preview button.

1. **Image settings:** allows for selecting the visualization of the image, what colors should be used for tracking, and the number of frames used to calculate background.
 - a. **Plot style:** Can be used to select the way the image should be shown on the screen and in the output. The following settings are available.

- (1) “original”: show image in its original form
 - (2) “fixed scale”: shows original image with the background subtracted. Fixed means that the brightness of the image is not changed, as opposed to option (4) below.
 - (3) “body + feet + tail”: Shows the body+tail and feet of the mouse as the program identifies them Shows body of mouse body and tail with reddish colors, and feet with orange-yellow-white colors.
 - (4) floating scale: showing original image but with brightness scaled to the full brightness range for easier visibility.
 - (5) “body+tail”: similar to (3), but only body + tail are shown.
 - (6) “body only”: similar to (3), but only body is shown.
 - (7) “feet only”: similar to (3), but only feet are shown.
 - (8) “tail only”: similar to (3), but only tail is shown.
 - (9) “all – no filter”: similar to (3), but the size-filter is not applied, i.e. areas that are outside of the required size range are shown.
- b. **body color / foot color / tail color**: the respective body parts will be identified using the thresholds within the color (R, G or B) defined here.
 - c. **Invert image**: inverts image for plotting as well as tracking purposes.
 - d. **Invert body / foot / tail**: inverts the respective body parts for tracking, i.e. the thresholds are inverted ($\text{inverted_threshold} = 255 - \text{threshold}$).
 - e. **BG frame #**: number of frames to be used to estimate background. Frames are taken at uniform distances within the whole image sequence to ensure that animals are not present at any location for the majority of the frames selected.
 - f. **body / foot / tail bright**: brightness setting for the different body parts. These values are multiplicative factors determining the brightness, i.e. doubling this number will double the brightness of a given pixel that is part of the body / foot / tail. Note that brightness can saturate, and 0 brightness will not change due to this factor.
2. **Track plot**: determines how tracking information should be shown in the images.

- a. **nose, head center, head contour, body center, body back, 1, 2, 3 tail, tail contour, feet:** these body parts will be shown in the images only if checked here.
 - b. **contour width:** thickness of the contour lines.
 - c. **foot marker size:** size of the circles drawn at the locations of identified feet.
 - d. **foot font size:** font size of letters identifying the feet.
 - e. **dir. bar length [pix]:** length of bars showing the direction of body parts.
 - f. **dir. bar width [pix]:** thickness of bars showing the direction of body parts.
 - g. **centroid size [pix]:** size of centroid drawn for each body part
 - h. **auto plot:** during auto tracking, the screen will be redrawn after each frame is tracked only if this option is checked.
 - i. **hide:** allows for the complete hiding of tracks, i.e. as if all the above options were unchecked.
3. **Filter:** Determines the range for each of the three color components within which pixels will be considered parts of the background. Take a pixel with median color (R_0, G_0, B_0) in RGB space, and assume that the Filter settings is (R_f, G_f, B_f) . For frame i , in which the pixel's color is (R_i, G_i, B_i) , the pixel will be considered part of the background if $|R_i - R_0| < R_f$ AND $|G_i - G_0| < G_f$ AND $|B_i - B_0| < B_f$.
4. **Tracking settings:** parameters specifying how to identify different body parts.
- a. **body min / max [0-256]:** brightness range within which pixels will be considered potentially parts of the body. This brightness range refers to the color component specified by "body color" in Image settings.

- b. **foot min / max [0 - 256]**: similar to a. but for footprints.
 - c. **tail min / max [0 - 256]**: similar to a. but for tail.
 - d. **min body size [pix]**: minimum number of pixels within a continuous area that will be considered a body.
 - e. **min foot size [pix]**: similar to d. but for footprints.
 - f. **min tail size [pix]**: similar to d. but for the tail.
 - g. **max tail width [pix]**: maximum thickness allowed for the tail.
 - h. **head length [pix]**: distance within which the head is identified as compared to the location of the nose.
 - i. **max body step [pix]**: maximum allowed displacement of the body between consecutive frames. The animal can't "jump" more than this per frame.
 - j. **max foot step [pix]**: maximum allowed displacement of a footprint between consecutive frames.
 - k. **min / max finger size [pix]**: minimum /maximum size of continuous area that can be considered a fingerprint (i.e. part of a footprint).
 - l. **max finger dist [pix]**: maximum gap between fingerprints to be considered part of the same footprint.
 - m. **fixed body length [pix]**: this distance will be considered as the true length of the body of the animal during evaluation. If its value is -1 then the program will use the median length of the animal, as reconstructed by the tracking. The tail is not counted in the length of the animal.
5. **forced direction**: The program aims to identify the front and back of the animal. For poor video quality, this may be difficult and the nose and hind point may be mixed up.

Specifying forced direction will make the program select the point that is in the specified direction, or by selecting “previous” it will select, for each frame, the one that is closer to the identification on the previous frame.

6. **Preview:** one can preview the how the changed setting affect the shown image without saving the new settings using this button.
7. **OK:** saves settings for this specific analysis.
8. **Save as default:** saves settings for this specific analysis AND saves settings as default for later analyses.
9. **Cancel:** quits without saving settings.
10. **Parameters:** specifies the frame rate of the image sequence (frame/sec) and the conversion between a pixel size and cm (pix/cm). The pixel to cm ratio can be calculated by taking a snapshot of a ruler on the walking floor and by measuring the distance in pixels of one cm using imageJ or Photoshop
11. **Boundaries [pix]:** the edges of each image specified by these four numbers will be removed from the analysis.
12. **Length bar:** if selected, a length bar will be shown on the screen for calibration.
13. **Default directory:** sets the default directory where the image sequence can be found and where the files generated by the program are saved. These values will be given to every newly analyzed video, or will appear every time one starts MouseWalker.

IV. Shortcuts

1-9	toggle between different plot styles (see manual)
v	jump to first frame
z OR <left>	decrease frame index by 1
x OR <down>	Select (load and plot selected frame index)
c OR <right>	increase frame index by 1
b	jump to last frame
e	toggle right fore leg
d	toggle left fore leg
q	toggle right hind leg
a	toggle left hind leg
r	toggle center of body
f	toggle head center
n	toggle nose
m	toggle tail end
t	cut out
g	ruler
E	set right fore footprint the same as it is on next frame
D	set left fore footprint the same as it is on next frame
Q	set right hind footprint the same as it is on next frame
A	set left hind footprint the same as it is on next frame

V. Footprint editing

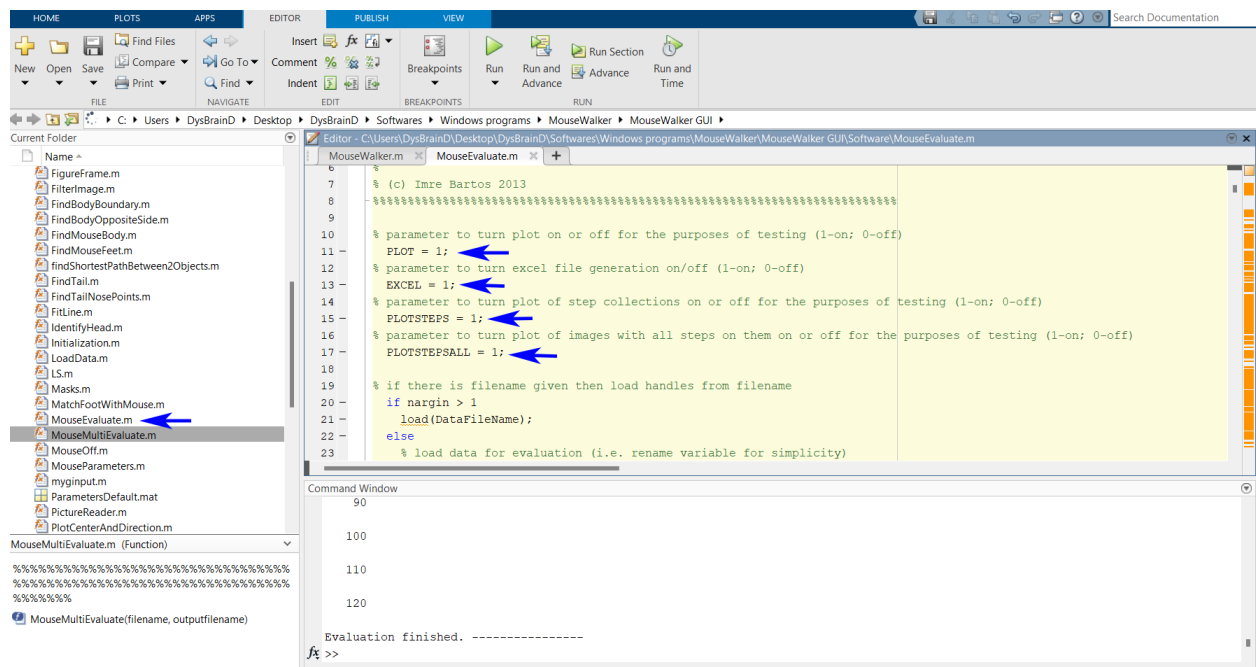
Once the auto tracing feature was completed, the user needs most of the times to correct mislabeled footprints. Footprints can be deleted or inserted by pressing the according button. If a footprint is deleted, then all successive (and continuous) footprints will also be deleted. Similarly, if a footprint is added it will stay in the subsequent frames until the same label reappears elsewhere.

If a footprint is absent in a particular frame but present in the precisely subsequent one, then the user can press the corresponding footprint key plus the Shift key. In this case the footprint will be placed precisely on the same location as the following frame. On the other hand, if the footprint is absent in a particular frame but present in the precisely preceding one, then the user can press the corresponding footprint key plus the Shift and Alt keys. In this case the footprint will be placed precisely on the same location as the preceding frame.

Pressing “Evaluate” will generate a large set of plots. By quickly inspecting these graphs, the user will realize if the tracking was correctly done, in particular if “lost” footprints were overlooked. In these situations correct the problem and re-evaluate the video.

VI. Quantification outputs

Since the evaluation process can be time consuming in order to generate all the output files (excel spreadsheet, plots, footprint images etc), the user can select what classes of outputs are generated. For this the user has to open and edit the MouseEvaluate.m file and change the outputs from 0 to 1 (see image). Naturally, the evaluation process will take longer if all output options are turned on (to 1). To verify if the videos are properly tracked the first option “PLOT” will suffice.



VII. Troubleshooting and tips

- The use of a scrolling device such as the ShuttleProV2 allows an easier scrolling between frames and execution of shortcut keys.
- In order to calculate the minimum area for the body and tail use the area measurement feature on imageJ
- To achieve the best footprint settings, check not only min intensity but also min area, switch between modes 1 and 3 (check section III.1.a.) to confirm if settings are correct
- If multiple auto-tracking sessions occur, make sure to overlap with the current mouse in order to prevent the appearance of a “new” mouse. If more than an untracked frame is present the script will assume this is a new mouse.
- The speed of scrolling and tracking between frames largely depends on the size (resolution) of each frame and the total number of frames of the tracked video. Thus make sure the video is cropped to the ROI and that only frames which will be tracked are present. Remove frames where the mouse is entering or exiting.
- In the case of movies saved as sequence files, the sequence file format should have the same amount of digits. For example, 100 after 099 and not 100 after 99.
- The output directory can be set to “<default>” so that is the same as the input folder.
- Frame scrolling will be faster in mode 1 and with no or minimum pixel boundaries.
- Before carrying out a set of video acquisition experiments, test if the light conditions for the walkway and background are properly detected on the MouseWalker.
