

Whisker Tracker Documentation

Description of this document:

This is a documentation file accompanying the Whisker Tracker (WT) software developed in the Ahissar Laboratory at Weizmann Institute of Science, Rehovot, Israel. For the latest version of the software and this document, contact Per Magne Knutsen (per.knutsen@weizmann.ac.il or pmknutsen@gmail.com).

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DISCLAIMER

The Whisker Tracker (WT) software is work-in-progress. So is this Documentation file. Some text contained herein may refer to previous versions of the software and may longer be valid. Furthermore, some sections do not contain complete information. This applies in particular to sections that have been highlighted in yellow.

Requirements

This software will run on any Windows compatible PC running Matlab version 6.1 or higher. Source code is provided should a user require the software to run on any other operating system (modifications will be required). A three-button mouse, or a two-button mouse with a wheel, is required for some of the functions in the software.

Installation

To install, unpack the distribution archive using WinZip or similar compression/decompression and then copy all directories and files to the desired path. It is not crucial, but advised, that the original directory structure is preserved. After installing the software, add the path to the WT directory in Matlab. All WT sub-directories should be added to your path.

To run the whisker tracker, type *wt* at the Matlab prompt.

Before you start tracking...

Note that WT makes a couple of assumptions about the movie you wish to process. Read therefore the following instructions before you start your experiments and before you attempt any tracking.

- i) Your movie must be in a format that is supported by MatLab. This includes various format containers such as AVI, and many different compression formats. However, as of writing, loading of QuickTime movies for instance is not supported by MatLab.
- ii) Your movie must have been acquired at a high-frame rate. WT probably won't succeed tracking movies less than frame rates of 100 fps, although you may use the manual tracking features to track movements in movies of any frame rate. We recommend you acquire your movies at 500 fps and up.
- iii) The rat's head should be parallel to one of the axis of the video frame. For instance, do not align the camera such that the rat is oriented 45 deg across the image. This is not a strict requirements, but if the rat wasn't properly oriented you will have to rotate the image later in WT. WT can rotate images by 90 deg quite fast, but it gets really slow at other angles. Of course, head orientation may be impossible to control in freely-moving animals. Thus, such movies may track slower than movies with head-fixed animals.
- iv) WT assumes that the video was taken from directly overhead the animal. Although WT may work with other camera orientations, it is not recommended you place the camera at large angles that deviate from a direct top-down view of the animal.
- v) WT assumes that the anterior direction is down in the image. It is not necessary to acquire videos this way, since WT can be instructed to rotate the frames. However, you must rotate the frames correctly in WT before you start tracking.

Tracking whisker movements of head-fixed and anesthetized animals

The following is a brief tutorial on how to the whisker movements of an anesthetized of head-fixed animal (basically, any movie where no head movements occur). To practice the following steps, you can load the supplied `demo_anesthetized.avi` demonstration movie from the `./WT_INSTALL/movie` directory.

Note that when you require to track bilateral whisker movements (i.e. whiskers belonging to both whisker fields) there is an additional step (# 5) that is not required when tracking unilateral whisker movements. If you track bilateral movements, you can skip step # 8.

- 1) Select *File->Load movie*
- 2) Locate the movie you wish to track on disk
- 3) Select *Image -> Select ROI* and draw a rectangle that includes the entire area covered by the whiskers. Include also a part of the whisker pad.
- 4) Select *Image -> Rotate clockwise* or *Rotate anti-clockwise* until the whisker protraction direction of the whiskers points downwards.
- 5) Bilateral movements only:
You must define the stationary position of the head. Select *Head->Set static head position* from the menu. When the cross-hair appears, click once on the right eye, once on the left eye and once on the tip of the nose.
- 6) Select *Whiskers -> Mark new whisker*. Decide on a whisker that is clear against the background. Click once on the whisker close to the whisker pad, once halfway along it and once close to its tip.
- 7) Repeat step # 6 for each whisker you wish to track.
- 8) This step should be skipped when tracking bilateral movements:
Select *Measure -> Set reference angle*. Click on two points that define the axis of a vertical line running from top to bottom of the frame such that the line will cross the whisker somewhere close to its root. Whisker angles will be measured relative to this line.
- 9) Click the > button in the menu to start tracking all marked whiskers. You can stop the tracking at any time with the || button in the main menu.
- 10) To display your tracking results, select *Whiskers -> Organize plots*. Select the parameters and whiskers you want to display.
- 11) To save your tracking results, press S on the keyboard when the WT main window is active, or select *Save data* from the *File* menu. The data is by default saved to the same path as your movie file.

Tracking head movements of freely-moving animals

WT tracks head movements by detecting the position where the light of an overhead spotlight reflects off the eye. It may be possibly to adapt WT 's head tracking routines to different scenarios, e.g. when two marker are attached to the skin close to the eyes or when the eyes are very small in the image. The reflections are manually located in the first frame of every movie. In the next and subsequent frames, a small region centered on the location of the eye in the previous frame is smoothed with a low-pass filter. Since movement of each eye from frame to frame is small, the location of the peak luminance indicates the new position of the eye. This procedure is repeated for all frames of the same trial until either eye disappeared out of the camera's view. Location of the nose is estimated as the vertex of an isosceles triangle with the base between the eyes. The base-nose angle is kept straight, and its distance constant, for all frames in each movie. For more details, see Knutsen et al (2005).

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Follow these steps to track the head-movements of an appropriate labeled animal. You can load and practice these steps on the demonstration movie `demo_freely_moving.avi` in the `./movies` directory where you installed WT.

- 1) The first step is to locate the position of the eyes manually in the first frame. Navigate therefore to the first frames in which the reflections are seen clearly on both eyes.
- 2) Select *Head -> Run head tracker* from the menu
- 3) The mouse pointer will be replaced by a cross-hair. Align the cross-hairs onto the left eye of the rat (right part of the frame), then click the left mouse button. Repeat for the right eye and the tip of the nose.
- 4) The head trackers will not complete tracking automatically until you press Stop.

Tracking whisker movements of freely moving animals

The routines used for tracking whisker movements of freely moving animals is very similar to that of tracking head-fixed or anesthetized animals. By freely-moving, we mean basically any movie where the head of the animal moves. This necessitates a correction for head-movement when we track the whiskers. Thus, the first step for tracking whisker movements in freely-moving animals is to track the head throughout the movie.

Start tracking by running the steps in the section [Tracking head movements of freely-moving rats](#) above. Once this has been done, the WT main window will automatically split into two panels. The left-hand panel will display the whisker field adjacent to the animal's left whisker pad, and the right panel the whisker field adjacent to the right whisker pad. To track a whisker, simply run the same steps as in the section [Tracking whisker movements of head-fixed and anesthetized animals](#) above.

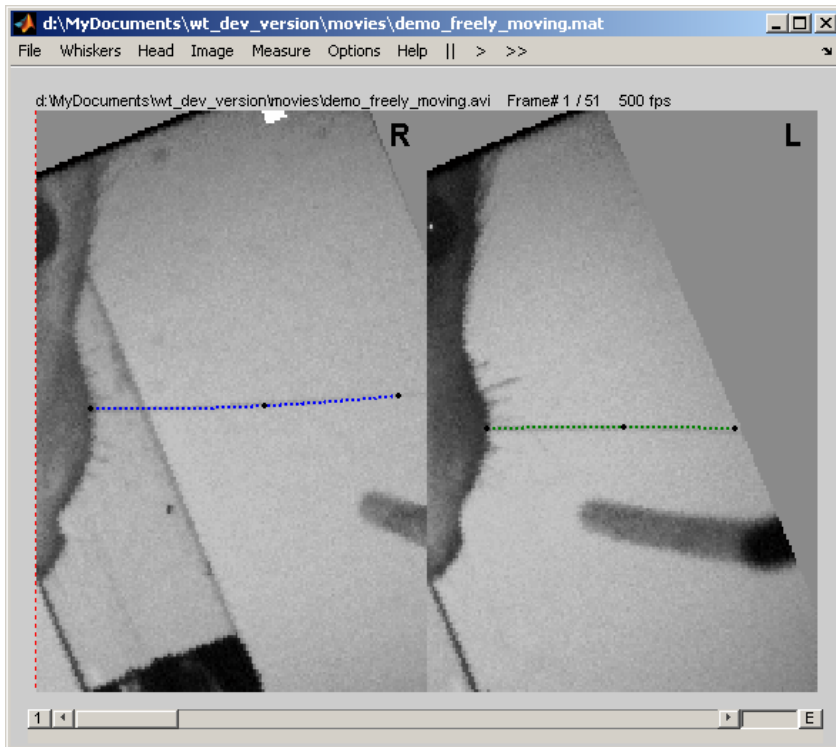


Figure 1.

Movie of a freely-moving rats with head and whiskers tracked.

Tracking labeled whiskers

Detecting object contact

For each whisker that you tracked you must now mark the location on the object where the whisker touched. If a whisker does not touch an object, you should still mark the most likely location the whisker would have touched if it had protracted further.

- C. Right-click on the whisker, and select "Add touch location"
- D. Align the cross-hairs and left-click exactly where the whisker touches the object.
- E. Repeat this for all tracked whiskers
- F. If the same whisker touches the object in different locations, at different times in the movie, add a second or third touch location the same way you added the first.
- G. If any mistakes are made, the touch location marker can be deleted or moved by right-clicking on the location marker and selecting "Delete" or "Move".

Each touch location is associated with a frame number, which is shown in white next to each touch location. For each individual frame of the movie, a whisker's position will be related only to a single object location. The frame number of the object indicates the first frame when the object location is the current reference. If another object has a higher frame number, then this object will be the reference from that frame and the object that was the reference up till that point will cease to be so.

It is important that when you mark multiple touch locations, each touch location should be associated with a frame number before the whisker actually contacted the object at that coordinate. The easiest way to achieve this is to use ProVideo to identify the frame where the whisker first touches at the new location, go to that frame in WT and mark the object and then set the associated frame number to a few tens of frames less than the current frame number (i.e. the frame you originally marked the object in).

Graphical User Interface (GUI)

The GUI has four main elements:

- 1) The [main menu](#) at the top of the window is accessible after opening a movie.
- 2) After tracking a whisker, options for controlling tracking of each individual whisker is available via a [context menu](#) that pops-up whenever you make mouse right-click on a whisker.
- 1) A [slider](#) appears at the bottom of the main window to move between frames. If you click on the slide itself, the movie will scroll 10 frame backwards or forwards. If you click on the arrows, the movie will move 1 frame at a time.
- 2) A status bar appears below the slider that informs you about errors and other useful information.

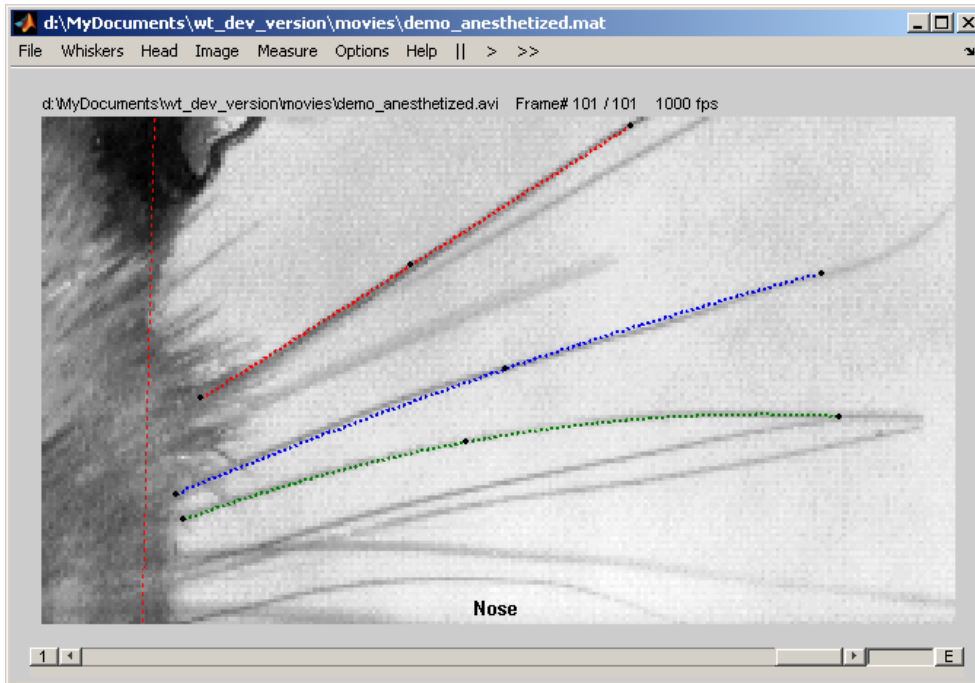


Figure 2.

The Whisker Tracker GUI. A movie of an anesthetized rat has been opened

Main menu commands

File

File -> Load movie

Click to load a single movie.

File -> Load directory

Click to load all movies in a directory. A list of movies will be generated that can be conveniently accessed in *File -> Movies*

File -> Load batch-file

Click to load all movies defined in a batch-file. The format of the batch-file must be:

PATH<TAB>START:STOP

Any number of such lines can be added to the batch-file. PATH is a common part of the entire file path that is shared between all files. For example, the directory path is common to all files that reside in the same directory. Also, a prefix common to all files may be included. START is a number that defines the first movie to be loaded. START will usually mean trial-number. END is the last trial to load. The following is an example that will load movies numbered 1 to 20 from directory f:\video\113\11-2-2003\1\ with the prefix c_

f:\video\113\11-2-2003\1\c_ 1:20

Note: Support for the batch mode has been discontinued in newer versions of WT. Thus, the batch mode may not function as originally intended. This option may be removed in future versions of WT.

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File -> Load Data

Load a specific *.mat* file that contains data from previous tracking sessions. You may use this feature to pre-load common settings, for instance.

File -> Save Data

Save whisker tracker data to disk. The data will be saved with a filename identical to the original movie's filename with the addition of the *_whiskers.mat* suffix (note that the separate head tracker application, *ht*, saves data with the *_head.mat* suffix). For instance, data for the movie *co08_096.avi* will be saved as *co08_096_whiskers.mat*. This file will be automatically saved in the same location as the movie.

The saved data-file contains a single structure with all data (spline-points, angle and intersection), in addition to all parameters used in the tracking. If parameters were changed during tracking, then the last used parameters will be saved in this structure. This command can also be accessed through the keyboard-shortcut *S*.

File -> Save Data As

Same as *Save Data* but allows you to save to a file with a different name and path than the open video file.

File -> Movies

Displays a list of all loaded movies. Clicking on a filename will prepare the workspace to start working on that movie.

File -> Movies -> Next in list

Clicking here will initiate the workspace for the next movie (from the current) in the list of loaded movies.

File -> Exit

Exits the whisker tracker.

Whiskers

Whiskers -> Mark new whisker

Displays the first frame in the current movie and waits for the user to click in three locations in the image. Each location defines a point along the whisker. A spline (an interpolated smoothed curve) is then drawn through these points. The points should be selected such that the spline more or less completely overlaps the whisker. Some general recommendations for selecting good spline-points are:

- Mark the points as far apart as possible, but avoid locations where the whisker is not clearly visible against the background (e.g. where there is a shadow or object below the whisker). Note, however, that it is generally ok for the spline to cross such regions.
- Avoid marking the extreme points close to the edges of the image, or close to fixed features in the image (such as the rat's head or an object).

If you are not happy about the spline you selected, delete it via the whisker's context menu (see below) and mark your points again.

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Tip: If you selected this option, but wish to abort, press RETURN or CTRL-C

When the whisker has been marked, a dialog will automatically appear requesting the name of the whisker to be entered. This step is optional. You may enter here the whisker identity (e.g. C1, alpha etc), or close the window without entering anything. The name is used only for identification purposes later in analysis or during graphing of kinematic parameters.

Whiskers -> Clear all whiskers

Clears ALL whiskers marked. It will also clear all information related to those whiskers, including angle and intersection. Saved information will not be cleared, unless you select *Save data* from the menu (see below).

Whiskers -> Delete whisker

Opens a small dialog where you can select to delete specific whiskers. Caution: using this option to delete whisker information deletes ALL tracked information about that (those) whisker(s)!

Whiskers -> Set last frame

Opens a dialog where the last frame to be tracked should be entered. When WT tracks automatically, tracking will stop once this frame is reached.

Whiskers -> Whisker display width

Set the width of the displayed whisker splines (default is 1 point). You can increase this number to make the whisker more salient.

Whiskers -> Show whisker identities

Enable this option to show the name of a whisker (e.g. C2) next to the displayed whisker spline.

Whiskers -> Continue tracking all labels

UNDOCUMENTED

Whiskers -> Adjust labeling filter

UNDOCUMENTED

Whiskers -> Mark whisker and label

UNDOCUMENTED

Whiskers -> Clear all labels

UNDOCUMENTED

Whiskers -> Use labels to track whiskers

UNDOCUMENTED

Whiskers -> Show names next to labels

UNDOCUMENTED

Whiskers -> Organize plots

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Opens a new window where kinematic parameters of tracked whiskers can be plotted. Whisker identity for each trace is available by the color-coding. The angle value is the whiskers angle relative to the reference line (calculated at the intersection).

Head

Head -> Run head tracker

UNDOCUMENTED

Head -> Set static head position

UNDOCUMENTED

Head -> Clean head movements

UNDOCUMENTED. For details, see [Filtering tracked movements](#).

Head -> Adjust eye filter

UNDOCUMENTED

Image

Image -> Select ROI

Select a region of interest (ROI). Allows you to limit the area that will be included in all the image processing. If the whiskers cover only parts of the image, select a rectangle that covers this area and no more. Limiting the region of interest will speed up the tracking process. Note that the spline-points that are later saved are relative to the region of interest, not the entire frame. Therefore, in order to reconstruct the whisker position (outside of the whisker tracker), you need information about the selected ROI. That information is available in the data structure described in *Whiskers -> Save data* above.

Image -> Toggle viewing mode

UNDOCUMENTED

Image -> Rotate clockwise

Rotates the image 90 degrees clockwise.

Image -> Rotate anti-clockwise

Rotates the image 90 degrees anti-clockwise.

Image -> Reference line

Mark a reference line that angle and intersection is calculated relative to. The reference line can be at any angle with respect to the head, such as parallel to the orientation-axis of the head or to the whisker pad. To mark the reference line, click on two points that will fall on this line. The reference line used will be drawn in red on top of the image. You can change the reference line by selecting this menu item again later. You can also recalculate all angle and intersection measure by selecting *Whiskers -> Plot angle* and *Whiskers -> Plot intersection* (see above).

Image -> Flip vertical

Flips the image over the horizontal axis.

Image -> Flip horizontal

Flips the image over the vertical axis.

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Image -> Refresh

Refreshes the currently displayed frame, updating whisker positions etc. Use this to recover the slider-bar at the bottom of the figure if it disappears.

Image -> Zoom

Allows the user to use the mouse pointer to zoom the displayed image.

Image -> Hide

Hides from view the video image but shows all tracked features and marked objects.

Image -> Return to default

Deletes all image manipulations and returns to the original image.

Measure

Measure -> Set reference angle

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Measure -> Set reference angle to default

UNDOCUMENTED

Measure -> Calibrate

UNDOCUMENTED

Measure -> Calibrate with imported image

UNDOCUMENTED

Measure -> Measure

UNDOCUMENTED

Measure -> Create outline

Allows user to draw/trace a static feature in the image, or to draw any object on top of the video image.

Measure -> Hide outlines

Hides all created outlines.

Options

Options -> Parameters

Opens a window in which parameters can be manipulated. Some parameters are also available in the menu (rotation, flipping etc) and others are not. See *The Parameters window* below.

Options -> Track stimulus triggers

UNDOCUMENTED

Options -> Scripts

WT can run your own functions that work on data in the currently loaded WT datafile. Your script should be formatted as a Matlab function, WT will pass a structure that contains all data in

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the currently loaded .mat file. You can use this feature to conveniently add your own extensions to WT. Your function should start with the following line:

```
function my_function_name(sStruct)
```

where my_function_name can be replaced by a name you choose and sStruct is the input structure that contains all WT data.

Options -> Show signal to noise

UNDOCUMENTED

Options -> Edit notes

UNDOCUMENTED

Options -> Show debug window

UNDOCUMENTED

Options -> Compress datafiles

UNDOCUMENTED

Options -> Uncompress movie

UNDOCUMENTED

Options -> Play movie

UNDOCUMENTED

Options -> Save movie

UNDOCUMENTED

Options -> Dump screen

UNDOCUMENTED

Help

Help -> WT Help

Opens this documents.

Help -> Keyboard shortcuts

Opens a window that displays a list of all available keyboard shortcuts.

Help -> About WT

Opens a window that displays the names of the programmers that contributed to WT, and copyright information.

//

Click here to stop tracking. Only applicable when tracker is active. You can easily tell of the tracker is active by noticing updates in the window where angle/intersection is plotted. Also, the background in the image window will turn green when the tracker is active.

>

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Click here to start tracking. You must select whiskers and a reference line before you can start tracking.

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Whisker context menu

The context menu pops-up whenever you right-click on a labeled whisker.

Track manually without deletion

This option allows you to track the chosen whisker manually from the current frame. The figure background will turn red when you are in manual mode and you should see a cross hair across the frame. Only the chosen whisker will be in color and all others will be black.

There are three possible operations in manual mode:

- | | |
|--------------------------|---|
| 1) Moving a spline-point | LEFT MOUSE BUTTON |
| 2) Jumping to next frame | RIGHT MOUSE BUTTON |
| 3) Exiting manual mode | MIDDLE MOUSE BUTTON or the > button in the menu |

To move a spline point, click on the new location for that point with the left mouse button. The spline point closest to that new point will be automatically moved to that destination. Note that the left and right point can only be moved up and down. The middle spline-point can be moved in any direction. When you are happy with the new shape of the spline, click the right mouse button to advance to the next frame. Click the middle mouse button or the || (Stop) button in the menu to exit from the manual mode.

Track manually with deletion

This option allows you to track the chosen whisker manually. Same as above, except that all tracking information related to this whisker in frames after the current one will be deleted before commencing manual tracking.

Clear from current frame

UNDOCUMENTED

Add point

UNDOCUMENTED

Remove point

UNDOCUMENTED

Constrain point

Select to constrain the radial (x-axis) movement of the middle spline-point. When the middle point is very close to either of the two other points, the risk of errors increase. If you see that the middle point often drifts toward one side, use this option to limit how far to the left or right the point can move.

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To limit the middle spline-point, click on the left mouse-button at the left-most radial position that will be allowed, drag the mouse over to the right-most radial position and release.

Move base-point

UNDOCUMENTED

Set last frame

UNDOCUMENTED

Add touch location

UNDOCUMENTED

Set full length

UNDOCUMENTED

Set whisker identity

UNDOCUMENTED

Mark whisker label

UNDOCUMENTED

Clean

UNDOCUMENTED

Delete

Select to delete the chosen whisker. This will also delete all data related to this whisker. Saved data is not affected unless you select *Whiskers -> Save data*. To delete all whiskers, select *Whiskers -> Clear all whiskers* instead.

Slider

You can click on the slider to move between video frames. Clicking on the arrows will move frame-by-frame. Clicking on the background of the slider will move 10 frames at a time. The current frame is always displayed in the title above the frame.

Clicking on the "1" button on the left side of the slider will change the displayed frame to the first tracked frame. Clicking on the "E" (E for End) button to the right of the slider will display the last tracked frame (with head or whisker movements).

The text field between the slider and the "E" button can be used to enter the frame number to be displayed directly (press Enter after the frame number).

Note you may also navigate between frames by pressing the G key and entering the frame number you want to display.

Occasionally, the slider may disappear. If this happens, select *Image -> Refresh frame* from the menu, or press the R key.

The tracking parameters dialog window

Display -> Rotate image (deg)

Degrees to rotate the image. You can rotate the image by any angle. However, only increments of 90 deg are recommended so other rotations are slow to compute.

Display -> Flip image (up-down left-right)

Enter two numbers either 1 or 0. Setting the first number to one flips the image up-down. Setting the second to one flips the image left-right. If you flip the image via the menu the numbers here will be changed as well.

Display -> Region of interest [x y width height]

Four points that define the region of interest relative to the dimensions of the original image. You can also set the ROI directly via the *Image -> Select ROI* menu item.

Display -> Interval between refresh (frames)

Number of trials between each time the angle/intersection plot and the whisker positions on the image will be re-drawn during tracking. Setting this number high will speed up the tracking.

Display -> Pre-load (frames)

Number of frames that will be loaded each time the whisker tracker loads frames from the current movie. The tracking process will be faster the higher this number is. However, the maximum number of frames is limited by computer memory. If you get error message such as “Out of memory”, try reducing this number (100-200 is a reasonable range that should work on most PCs).

Coordinates of reference line -> Point A

First coordinate of the reference line.

Coordinates of reference line -> Point B

Second coordinate of the reference line.

Whisker tracking parameters -> Horizontal jitter [left mid right] SLOW

Should contain three numbers that denote the horizontal jitter of each spline-point, in the order left, middle, right. These numbers should generally be low (1-3). A guideline is to observe in your set of movies for how many pixels the whiskers move at maximum velocity. If you set the numbers too low, the tracker will not be able to catch up with fast moving whiskers. If the values are too high, however, there is a good chance it will catch other nearby whiskers with similar shape. A value of 1 or 2 for the left point and 2-3 for the middle and right points seem to be optimal in those cases we have tested. The same set of values can usually be used across a series of similar movies where camera settings were the same. To use these Horizontal Jitter parameters, click on the slow 'Play' button in the WT main menu ('>').

Whisker tracking parameters -> Horizontal jitter [left mid right] FAST

This is an option set of Horizontal Jitter values to use when the whiskers are moving fast in the video. Since there is a tradeoff between how large horizontal jitter to use and tracking speed, you should always try to use the smallest possible values for the horizontal jitter. You can define two sets of such values, either SLOW or FAST. Use SLOW when the whisker is moving slowly, and

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the FAST values when the whisker is moving fast in the video. To use the FAST Horizontal Jitter parameters, click on the fast 'Play' button in the WT main menu ('>>').

Whisker tracking parameters -> Radial jitter of mid-point

One number that denotes the radial jitter (i.e. along the whiskers) of the middle spline-point. Note that only the middle point can move along the whisker. The other two points are fixed and can only move in the horizontal (whisking) direction.

Whisker tracking parameters -> Whisker filter width

An estimation of typical whisker width in pixels. If you are uncertain, increase the magnification and count how many pixels wide the whiskers are. A value of 1 or 2 is typical.

Whisker tracking parameters -> Width of local-angle filters

The radial length of the filter. A value around 10 is recommended in most cases.

Image pre-processing -> Background frames [from to]

An average of selected frames can be subtracted from each frame before localizing the whiskers. This will generally remove stationary objects in the movie, such as the rat itself and non-moving objects. The field should contain two values, where the first denotes the first frame to start averaging from and the second number is the last frame to average to. The frame numbers should be entered like this:

[1 500]

If you do not wish to subtract an average image, then set both numbers to zero:

[0 0]

Entering only a starting and end frame will result in the background frame only being subtracted from the *same frames*. To subtract the background image from *all* frames, add two zeroes before the frame-range, separated by a semi-colon, like this:

[0 0; 1 500]

You may also compute background frames for multiple ranges, and then average these:

[0 0; 1 500; 1000 501]

If you enter the same numbers without the two zeros, then each background frame will be subtracted only from the *same frames* as it was derived from.

Image pre-processing -> Invert contrast

If a value of 1 is entered here, the image contrast will be inverted before tracking.

Position extrapolation -> Enable (0/1)

Set this field to be 1 to attempt extrapolating the most likely position of the whisker in the next frame given the position of the same whisker in all preceding frames. The result of this operation is unlikely to be very accurate, as it is sensitive to local changes in whisker velocity. Using this option may slow down tracking.

Position extrapolation -> Scale filter by factor of

The half-width of a gaussian (in pixels) used to smear the bias contributed by the extrapolation bias. As the extrapolation may in some cases be exaggerated, setting this value low will almost invariably cause an error at some stage during tracking. A large value, say about half of the height of the cropped image (i.e. after selecting the region of interest) is recommended. If uncertain, use a value of 100.

Angle calculation -> Pixels from base

The angle of the whisker is calculated relative to the reference line between two points: 1) the point where the whisker crosses the reference line, and 2) a point located X points radial from the first point. The second number is set in this field and is in pixels.

Calibration -> Length of calibration bar (mm)

The value in this field is automatically inserted when you calibrate the movie (see [Calibration](#) section). You can manually adjust it here if necessary. The unit of the length of the calibration bar is millimeters.

Calibration -> Calibration marks [Xa Ya; Xb Yb]

The values in this field are automatically inserted when you calibrate the movie (see [Calibration](#) section). You can manually adjust them here if necessary. These values are the X and Y coordinates of the two calibration points you selected during calibration.

Filtering tracked movements

WT includes a feature to smooth tracked head and whisker movements. To smooth tracked head movements, enter the smoothing window via the menu item *Head -> Clean head-movements*. To smooth tracked whisker movements, right-click on the whisker and select *Clean* from the [whisker context menu](#).

Important: The smoothing procedures described next apply filters to the raw, tracked data. These smoothing operations modify the actual coordinates of the tracked points, and are therefore irreversibly unless you first make a backup of your data.

To clean either whisker or head movements, open the filtering dialog window (see above). Select your filtering parameters (see below for descriptions of the 3 parameters available). When you have selected your filtering parameters, the new filtered trace (blue) will be plotted on top of the old unfiltered trace (red). Note that each sub-panel in the windows corresponds to the tracked coordinates of a single point either on the X or Y axis.

The following is a description of the filtering dialog options:

Width and Amplitude

The filtering procedure can apply a special filter to the data. Because of the pixelization of the video images, you may notice in your tracking (especially if the movies have low spatial resolution) that the tracked points jump between discrete levels. This filtering routine is optimized to detect and remove when a tracked point briefly jumps to a new level. The Width parameter sets the duration (in frames) and the Amplitude parameter the size (in pixels) of such brief jumps.

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Low-pass

This is the cutoff frequency (Hz) of a Butterworth low-pass filter applied to the tracked data. If no value is entered, the low-pass filter will not be applied.

Save changes

Saves the your changes. Note that saving is an irreversible action. The only way to retrieve your old, unfiltered data, is by tracking anew.

Exit

Exit the the filtering dialog. If you exit before saving your changes they will be discarded.

Labeling filter dialog

UNDOCUMENTED

Organize plots dialog

This dialog can be accessed from WT's main menu in *Whiskers* -> *Organize plots*. The dialog contains three sections: 1) a list of kinematic parameters that can be plotted for each whisker, 2) an option for applying a low-pass filter to computed parameters, and 3) a list of tracked whiskers along with their identifiers.

In order to plot one or several kinematic parameters for a particular whisker, or whiskers, select the parameter(s) and whisker(s) to plot by clicking in the corresponding checkboxes.

The 'Low-pass' option contains a single input field. This field indicated the cut-off frequency of a low-pass filter applied to all the displayed kinematic parameters. If no value is given, the low-pass filter will not be applied. Note that the cut-off frequency cannot exceed $(FPS/2)-1$, where FPS is the sampling rate of your movie (in frames/second).

The following is a list of explanations for each of the kinematic parameters.

Angle, Velocity and Acceleration

The angle, angular velocity and angular acceleration of the whisker. The velocity and acceleration are simply the 1st and 2nd derivative of the angle, respectively.

Curvature

UNDOCUMENTED

Reaction angle

UNDOCUMENTED

Integrated curvature

The integrated curvature is the integrated difference between a straight line and the shape of the whisker shaft. Thus, if the whisker shaft appears as a perfectly straight line in a frame, then the integrated curvature equals zero.

Kinetic energy

UNDOCUMENTED

Intersect

This is the y-coordinate of the innermost (most proximal) spline point. The unit is pixels.

Position from object

UNDOCUMENTED

Triggers

UNDOCUMENTED

Avg cycle (trig A)

UNDOCUMENTED

Avg cycle (trig B)

UNDOCUMENTED

After selecting the whiskers and kinematic parameters to plot, the selected graphs will be automatically be plotted in a new window. If the plotting window is already open, select *Refresh* in the menu to add new kinematic parameters or whiskers. Note that all kinematic parameters are plotted in the same Matlab figure panel. Thus, the timescale is common to all graphs but the y scale is arbitrary. To view both scales of a particular graph, right click on the graph and select *Copy to new figure* from the menu that appears.

The plotting window contains several additional options, accessibly by button presses:

G

Selecting this option will add a grid to the plotting window.

M

Selecting this option will add a marker (small dot) to every data point.

E

Selecting this option will export the data contained in the plotting window to a new Excel datasheet.

Average cycles

UNDOCUMENTED

Keyboard shortcuts

Several functions within WT can be conveniently accessed by a single key-press. The available keyboard shortcuts can be displayed in the *Help -> Keyboard shortcuts* menu.

P	Open parameters window
D	Dump current view to printer, file or clipboard
S	Save data
N	Load next movie in list
H	Hide/unhide image

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M	Play movie from current frame
SPACE	Stop/start tracking
Any number	Go back N frames. Pressing 0 will go 1 frame forward.
0	Go forward one frame
G	Open Go-to-frame dialog
E	Edit notes
Q	Go to first frame
W	Go to last frame whiskers were tracked to

WT Scripting

After, or during tracking, you will want to work on the tracked movement data. There are two main ways of accessing the data: i) you can either access the .mat files saved by WT directly from the disk, or ii) you can access the tracked data from within WT. The latter option is useful if you want to modify or add information to the tracked data, or for quickly visualizing new parameters that you compute from the data.

For this purpose, WT supports scripting. Scripting is a convenient feature of adding your own functionality to WT. The steps for adding a script in WT are simple:

- i) Write your extended function
- ii) Copy the function to the ./scripts directory where WT is installed
- iii) Run WT (of if WT is already running, press R to refresh the menu)
- iv) Run your script from the menu item Options -> Scripts

Your extended function should be formatted as a regular Matlab function and is written in Matlab syntax. When its run from within WT, it will receive a structure that contains all data in the currently loaded .mat file. Therefore, when defining your function, use the following form:

```
function my_function_name(sStruct)
```

where my_function_name is replaced by your function's name, and sStruct is the input structure that contains your tracked data. WT does not expect any parameters to be returned. For example, see the existing functions in the ./scripts folder.

Calibration

UNDOCUMENTED

References

The following is the original reference where the procedures in the Whisker Tracker software were first described. Please cite this reference whenever the WT software is used:

Knutsen PM, Derdikman D, Ahissar E (2005) Tracking whisker and head movements in unrestrained behaving rodents. J Neurophysiol 93:2294-2301.

The following are references where the Whisker Tracker has been used:

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