

FIMTrack v.2 Manual

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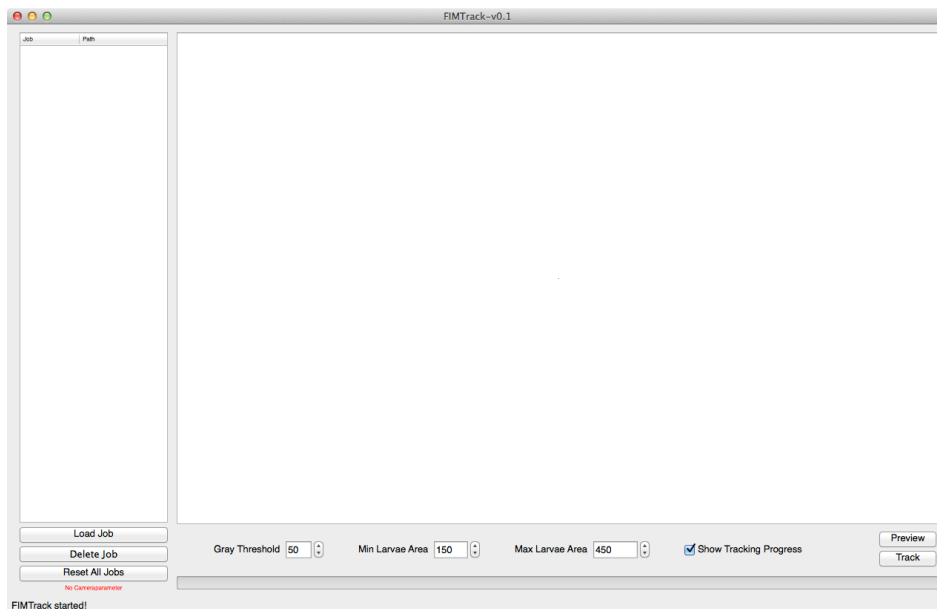


Figure 1: Main GUI of FIMTrack.

1 Introduction

FIMTrack is a larval tracking program to acquire locomotion trajectories and conformation information of *Drosophila melanogaster* larvae. It is optimized for FIM images. FIM is an acronym for FTIR-based Imaging Method, whereby FTIR is the short form for Frustrated Total Internal Reflection. Details can be found elsewhere [2, 1].

In this document, the basic functionality of the FIMTrack tracking software is described. The focus is on the *how-to* use the software. Furthermore, an overview of all calculated features and parameters is given.

2 Installation Instructions

FIMTrack is available for all operating systems, including Windows, Mac OS and Linux. If precompiled executables are downloaded, just start the program by selecting the executable FIMTrack file.

FIMTrack is written in C++. To manually compile the program, several additional (open source) libraries need to be installed:

- The OpenCV library
- The Qt library

FIMTrack uses Qt graphical user interfaces, thus all used libraries are listed in the `FIMTrack.pro` file. We recommend Qt Creator for development.

3 How To Use FIMTrack

3.1 How To Track

After starting FIMTrack the main GUI is displayed (Figure 1). To track larvae imaged using FIM imaging, simply select the TIFF-images by using the *Load Job* button (Figure 2).

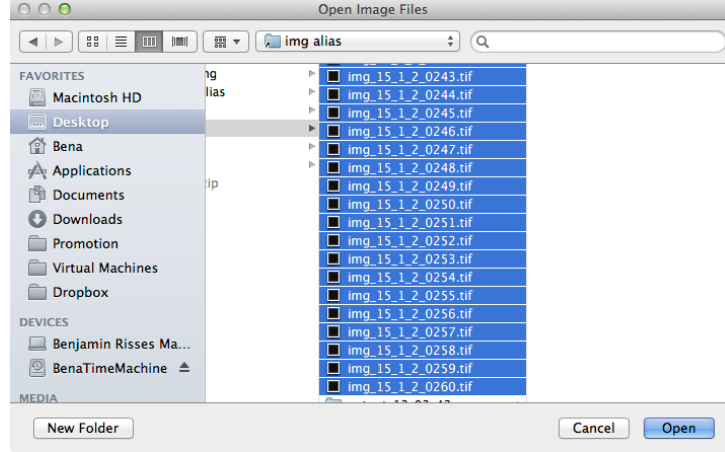


Figure 2: Select the images to be load into FIMTrack.

IMPORTANT: FIMTrack can only be used with TIFF-images. If the images are not available in TIFF format, conversion can be done using Fiji, ffmpeg or other third party software.

You can get a preview of the images by selecting images inside the loaded job (Figure 3). Navigate in the preview image by using the sliders around the image. You can zoom in and out by using *cmd* (or *Ctrl*) + *mouse wheel* or for faster zooming *cmd* (or *Ctrl*) + *shift* + *mouse wheel*. The preview is useful to set the following parameters given in the bottom of the main GUI:

- **Gray Threshold** specifies the minimal brightness of the foreground objects (i.e. larvae). If no larval foreground object is highlighted in the preview image, the Gray Threshold must be decreased.
- **Min Larvae Area** specifies the minimal area (in pixel) a larva must cover
- **Max Larvae Area** specifies the maximal area a (single) lava can cover.

Larvae are detected if their contour is highlighted blue in the preview image. Red, yellow and green circles serve as additional markers for correct detection. The blue number next to the larva indicates the covered area of the larva (in pixel; Figure 4a) to approximate the Min and Max Larvae Area. Refine the Min and Max Larvae Area threshold based on the blue area values given in the preview. **It is important that the Max Larvae Area is smaller than two colliding animals.**

If the larvae are smaller than the Min Larvae Area value, reduce this value and click on an image again to refresh the preview. Detected objects which are bigger than the Max Larvae Area value are indicated by red contours (Figure 4b).

The preview also highlights coiled larvae (i.e. the head is touching the tail). These animals are marked by purple circles inside the contour (Figure 4c).

3.2 How To Set Basic Preferences

Basic preferences can be adjusted by selecting the *Preferences...* option in the menu (Figure 5a). Before the tracking is started, adjust the scale factor and the frames per second rate in the basic preferences panel. The scale factor specifies the conversion from pixel to cm (unit $\frac{\text{pixel}}{\text{cm}}$) and the frames per second are given by the image acquisition rate of the camera.

3.3 Set Region Of Interest

You can set an arbitrary number or regions which should be included into tracking (Figure 6). Everything outside these regions will be neglected.

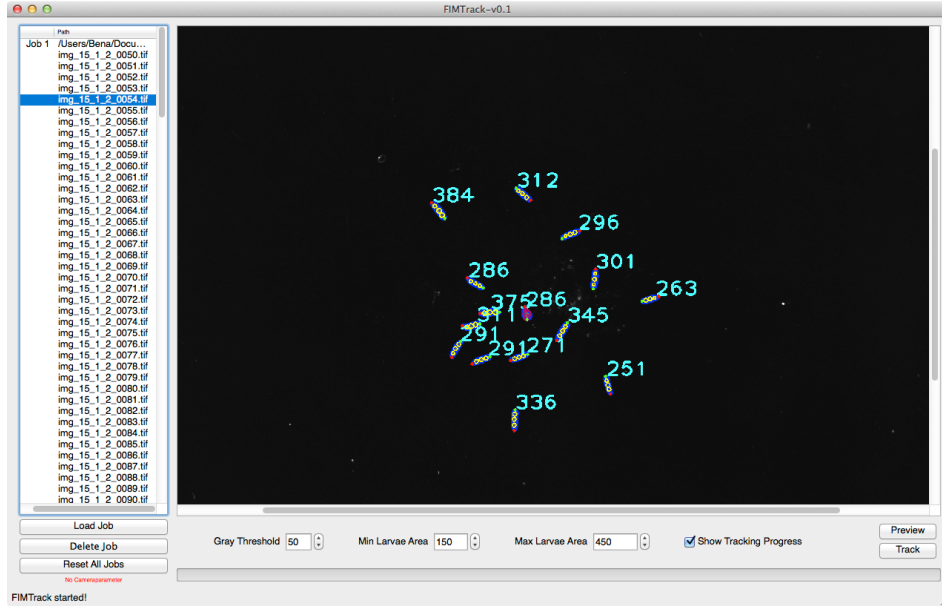
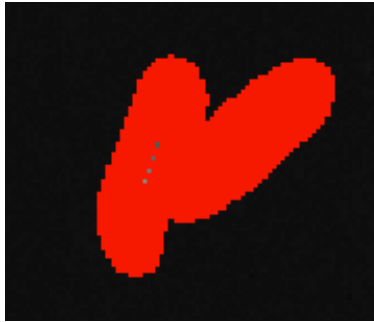


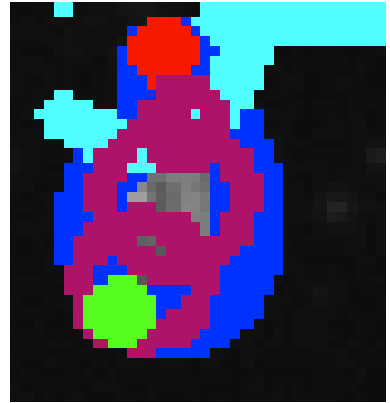
Figure 3: Preview image.



(a) Correct detected animal and its covered area.

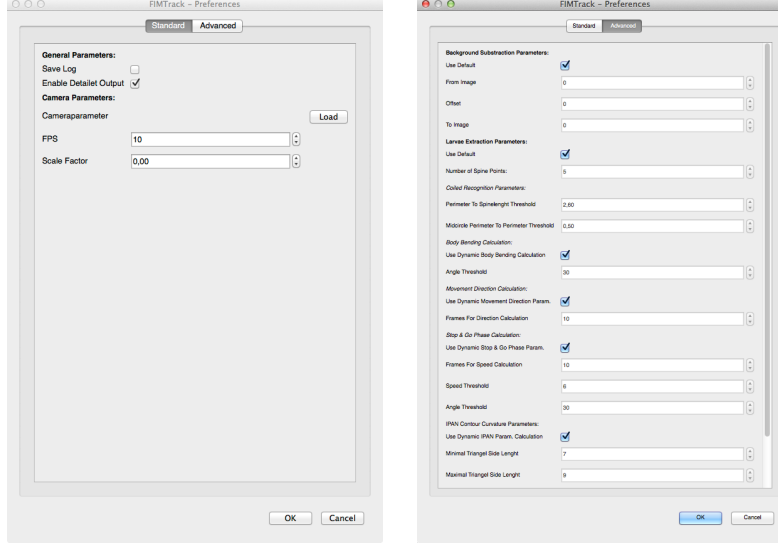


(b) Two colliding larvae exceeding the Max Larvae Area threshold.



(c) Coiled larva (i.e. head is touching the tail).

Figure 4: FIMTrack preview clippings.



(a) Standard preferences dialog. (b) Advanced preferences dialog.

Figure 5: FIMTrack preference panel

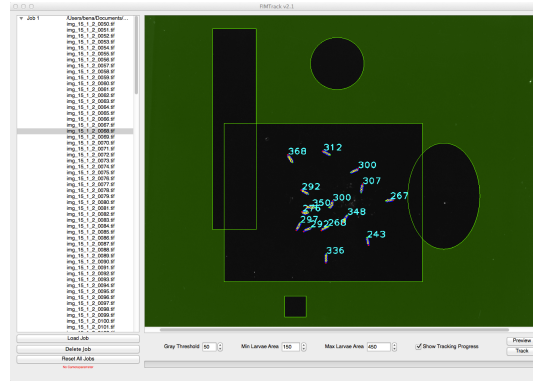


Figure 6: Several regions of interest are set.

A region of interest is set by right clicking in the preview window. Select the shape of your preferred region (rectangle or ellipsoid). You can move the region around by right clicking on the region and using the mouse. The region can be resized by selecting the lower right corner of the region (use *cmd* on Mac or *Ctrl* on Windows to highlight the corner). You can change the colour of the regions by double clicking in the region.

3.4 Start Tracking

If the parameters are set up sufficiently, hit *Track* to start tracking. The Tracking process is highlighted as long as the *Show Tracking Progress* check box is selected. **If nothing happens for the first seconds after clicking Track, this is because of the background image calculation, which is done before actual tracking (see message in the lower left of the main GUI).** If the tracking is done, a short message will be given in the lower left of the main GUI.

3.5 How To Adjust The Preferences

Preferences can be adjusted by selecting the *Preferences...* option in the menu. There are two types of preference panels:

- **Standard** preferences including camera related parameters (i.e. the frames per second (fps) used for tracking) and a scale factor to scale from pixels to meaningful units (Figure 5a).
- **Advanced** preferences include all tracking and feature extraction related parameters (Figure 5b).

Standard Preferences

The frames per second (fps) as well as the scale factor are important to calculate features independent of the used resolution. For example, the velocity is given in $\frac{mm}{s}$. The temporal normalisation is done using the fps rate and the conversion from pixel to mm is done using the scale factor. To set the scale factor, make an image of ruler and count the pixel within one cm (using Fiji or Photoshop etc.).

Advanced Preferences

The advanced preferences are structured in groups. Each group can be edited by unchecking the *Use Default* or *Use Dynamic ... Calculation* check box.

- **Background Subtraction Parameters** to calculate a background image without the animals.
 - **From Image** specifies the first used image for Background Subtraction
 - **Offset** specifies the number to increment the current frame to the next frame
 - **To Image** specifies the last image to use for Background Subtraction.
- **Larvae Extraction Parameters** for larval feature calculation
 - **Number of Spine Points** specifies the number of points placed along the spine. This value must be odd to guarantee a real mid-point.
 - **Coiled Recognition Parameters** to detect coiled structures.
 - * **Perimeter To Spinelength Threshold** is used to find coiled structures. A larva is in a coiled structure, if the head touches the tail so that the program cannot find distinct head- and tail points (Figure 7b). In this situation, the ratio between the perimeter (P in Figure 7b) and the spine length (L in Figure 7b) is similar to π (Figure 7a). Thus this ratio must be above this threshold ($\frac{P}{L} \geq \tau$).
 - * **Midcircle Perimeter To Perimeter Threshold** is used in a similar fashion like the Perimeter To Spinelength Threshold. If the ratio of the radius measured at the middle spine point (r) and the overall perimeter (P) is bigger than a given threshold κ the animal is assumed to be in a coiled structure (Figure 7c).
 - **Body Bending Calculation** to detect head sweeps.
 - * **Angle Threshold** specifies the curvature aberration (in both directions) to be detected as a head-sweep / bending (in degree)
 - **Movement Direction Calculation** to interpolate the movement direction calculation over time.
 - * **Frames For Direction Calculation** given high frame rates (e.g. 10fps), the movement detection cannot be calculated between consecutive frames since the moved distance between consecutive frames is too short. Thus the direction is calculated for every frame by comparing the current frame i and the frame at time $i + offset$, whereas this parameter specifies *offset*.
 - **Stop & Go Phase Calculation** to classify stop and go phases

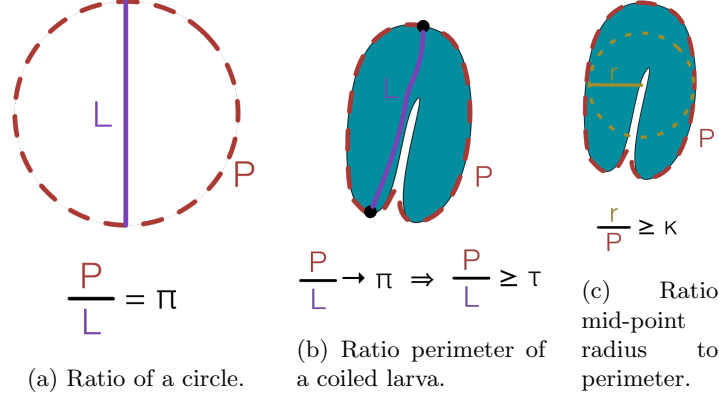


Figure 7: Coiled recognitions parameter: ratio perimeter (P) to diameter/larval length (L) is given for a circle and a coiled larva. The ration of the middle point radius (r) to the perimeter is sketched on the right.

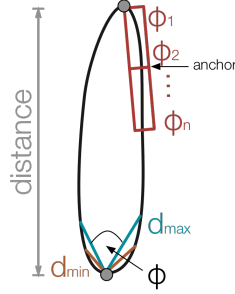


Figure 8: Parameters of the IPAN algorithm to calculate the spine (see text).

- * **Frames For Speed Calculation** given high frame rates (e.g. 10fps), the correct speed cannot be calculated between consecutive frames since the traveled distance between two consecutive frames is too small. Thus, an offset is used to calculate the speed between frame i and $i + offset$, whereas this parameter specifies this *offset*.
- * **Speed Threshold** given the speed calculated the offset from above, this threshold specifies the minimal speed a larvae must travel during consecutive frames.
- * **Angle Threshold** assuming that strongly bended animals cannot be in a go phase, this threshold specifies the abortive angle which is acceptable to be in a go phase.
- **IPAN Contour Curvature Parameters** to calculate the spine in general. The IPAN algorithm is used to calculate the curvature along the contour (Φ in Figure 8). Therefore, a triangle is spanned at each contour point.
 - * **Minimal Triangle Side Length** specifies the minimal length of a triangle lines (in pixel; d_{min} in Figure 8).
 - * **Maximal Triangle Side Length** specifies the maximal length of the triangle lines (in pixel; d_{max} in Figure 8).
 - * **Maximal Curvature Points Distance** specifies the distance the head and tail point must be apart (in pixel; distance in Figure 8).
 - * **Curvature Window Size** is used to smooth the calculated curvatures. The window size n is used to calculate the mean curvature at the anchor point (Figure 8).



Figure 9: Several jobs are loaded for batch processing.

3.6 Batch Processing

If all movies are captured with the same FIM settings, batch processing can be used to track an arbitrary number of movies. Just add as many jobs as necessary using the *Load Job* button (Figure 9). Single jobs can be removed from the list using the *Delete Job* button. *Reset All Jobs* removes all jobs from the list.

3.7 Output Of FIMTrack

After tracking, FIMTrack stores several files in the respective image folder. The naming of the folder is *output time* where *time* is the current time. FIMTracks outputs:

- **table.csv** excel sheets containing all calculated features.
- **output.yml** YAML files containing all features and additional tracking informations (used in FIMTrack Results viewer; see below).
- **tracks.tif** an image showing all detected trajectories. The spine is plotted for all animals and time points so that head sweeping can be seen in these images.
- **tracksNoNumbers.tif** similar to tracks.tif but without larvae IDs

3.8 View Results In FIMTrack

FIMTrack offers the possibility to review the calculated tracking results. It is also possible to adjust the tracking results or to manually track several animals. Select *Edit > Results viewer...* to open the Results Viewer dialog (Figure 10). Click on *Load Results...* to select both, images and tracking results. You can load tracking results by selecting **the same images** used for tracking (Figure 11). Then you are asked to select the calculated tracking results; i.e. the *output.yml* file (Figure 12). **The selected images must be exactly the same to the images you used for tracking. You can review the image names used for tracking in the output.yml file.**

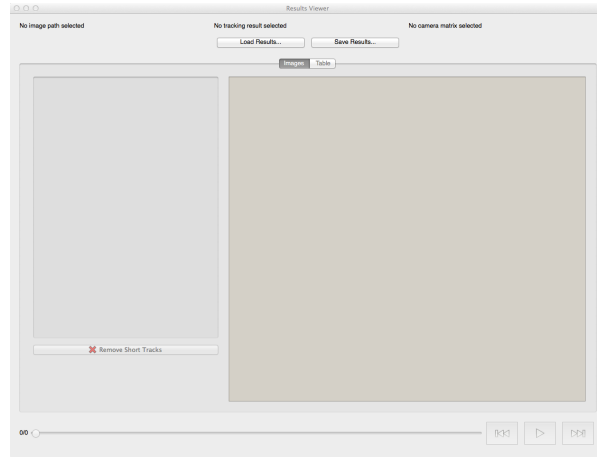


Figure 10: Results viewer GUI dialog.

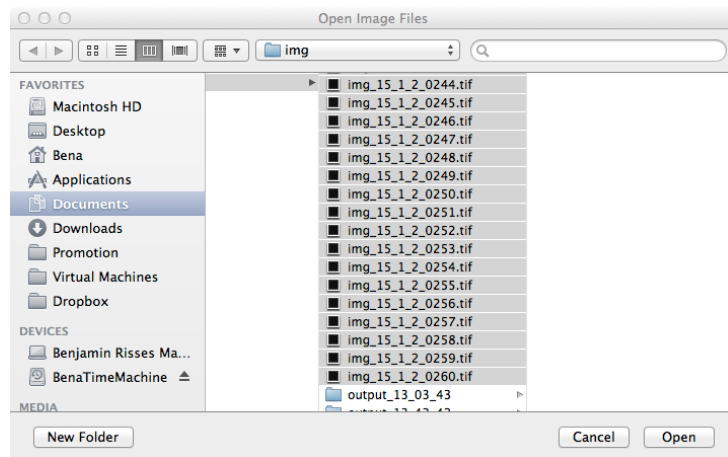


Figure 11: Select the same images used for tracking

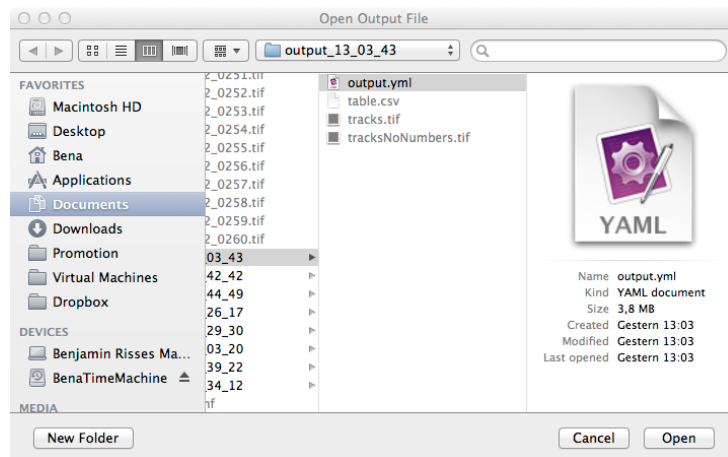


Figure 12: Select the calculated *output.yml* file.

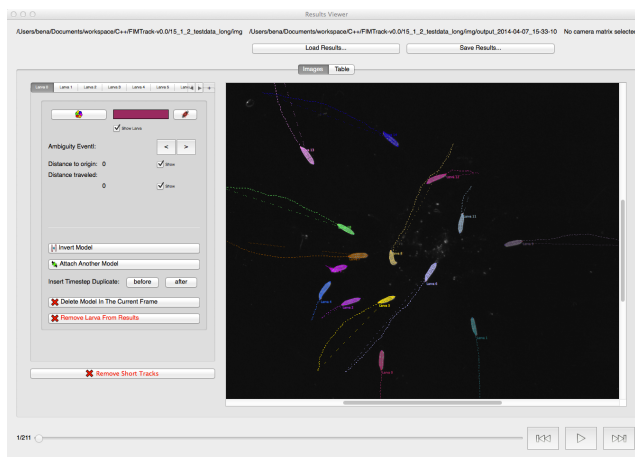


Figure 13: Image with plotted tracking results.

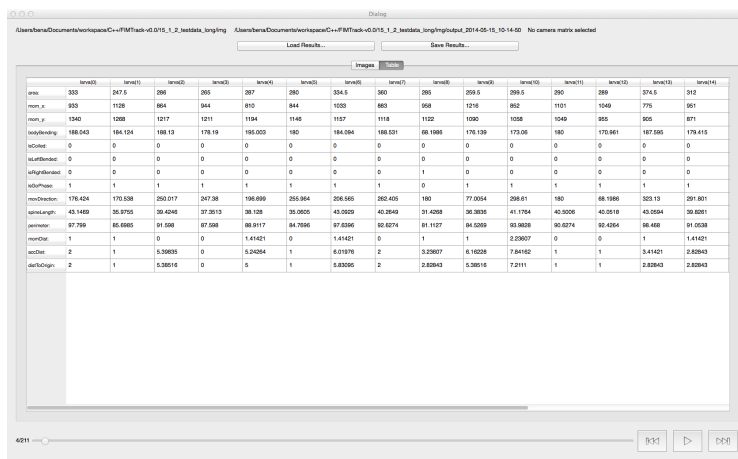


Figure 14: Current calculated features.

Using the Results Viewer

The results viewer has a *Image* and a *Table* tab to view the images and calculated features respectively. The image tab plots the calculated results onto the images (Figure 13). You can zoom in and out by using *cmd+mouse-wheel* or *cmd+shift+mouse-wheel* (Mac OS) or *Ctrl+mouse-wheel* or *Ctrl+shift+mouse-wheel* (Windows). In the table tab you can review all calculated features for the current larvae and time point (Figure 14). Push play or use the slider to step through time. All currently detected animal trajectories are drawn in the image tab of the results viewer (Figure 15). You can also use the left and right button to step from frame to frame (Figure 16).

3.9 Manual Tracking

In the larva tap you can adjust you tracking results.

The Manual Tracking Panel

The panel offers the following options (Figure 17):

1. Tabs to select the larvae

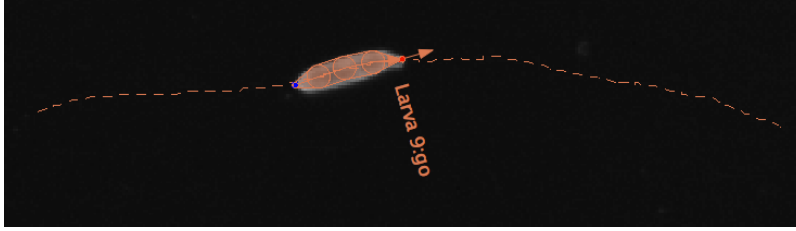


Figure 15: Current calculated features.

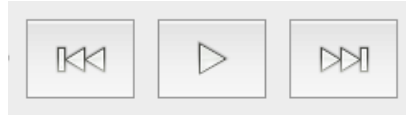


Figure 16: Step from frame to frame or select play to get a continuous preview.

2. Left and right button to scroll through the larva tabs
3. Change the colour of the larvae
4. Locate the larva
5. Checkbox to hide/show this larva
6. Jump to previous (left arrow) or next (right arrow) ambiguous time point of this larva (e.g. animal is lost because of a collision).
7. Current distance to origin and checkbox to draw this distance into the image.
8. Current distance traveled and checkbox to draw this distance into the image.
9. Invert the model (swap head and tail). The *inversion dialog* will appear (see below).
10. Attach the selected larva to another animal to unite the trajectories. The *attach to dialog* will appear (see below).
11. Insert a duplicate of this larva *before* or *after* the current frame
12. Delete model in the current frame (i.e. the larva at this specific time point).
13. Remove larva from results will remove all measurements from the selected larva.
14. Remove short tracks can be used to remove larval trajectories button. The minimal length in frames must be given in the remove larva dialog (see below).

Invert Larval Model

If the head and tail is swapped, this can be changed using the invert larval model button (9 in Figure 17). The button will open the invert larva dialog (Figure 18). It is possible to invert the current frame or all frames *Before* or *After* the frame selectable in the drop down menu below.

Attach Another Model

If two trajectories need to be united, the *Attach Another Model* button must be used. Select the model which should be attached to the current larva (Figure 19).

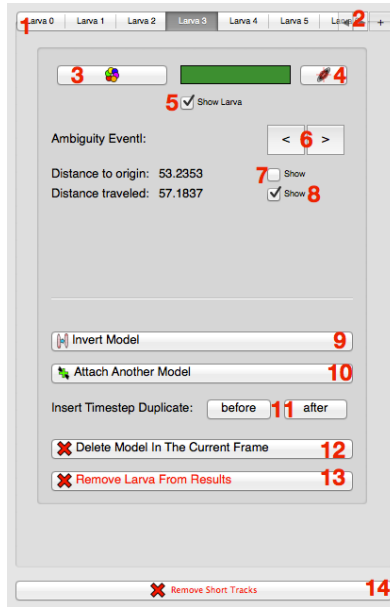


Figure 17: Edit trajectories panel (see text).

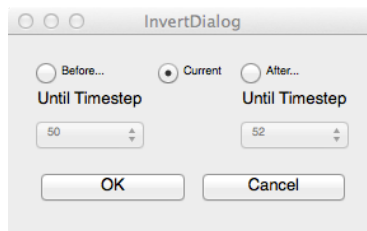


Figure 18: Invert a model of a larva.

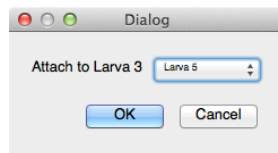


Figure 19: Select the larvae which need to be united with the current larva.

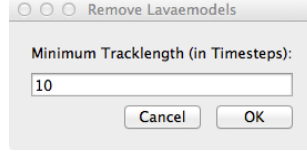


Figure 20: Dialog to remove short tracks.

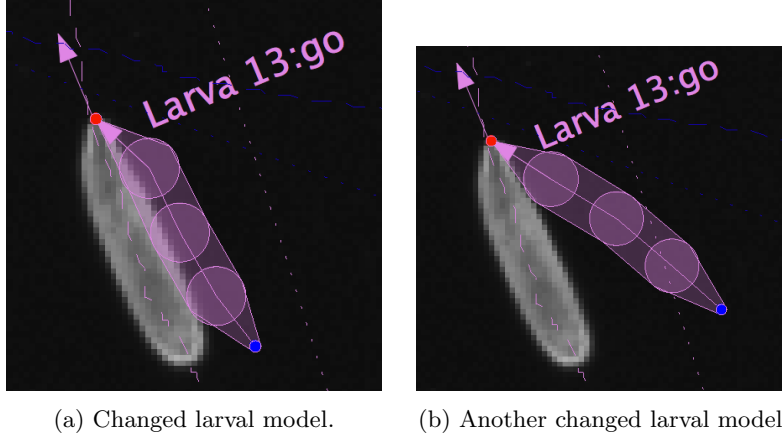


Figure 21: Examples of adjusted larval models.

Remove Short Tracks

Trajectories which are too short can be removed by the *Remove Short Tracks* button. In the following dialog, the minimal number of frames for a valid trajectory must be entered (Figure 20).

Adjust Single Larva

You can also adjust the posture and trajectories for each animal (Figures 21a and 21b). First select the larva tab of the animal you want to adjust (see 1 in Figure 17). Then select the point you want to change in the results image using the left mouse button. Hold the mouse button and move the mouse to change the location of the selected spine point. You can also change the size of the inner spine points (not of the head and the tail) by selecting the points (left mouse button) and pressing the *cmd* (Mac) or *Ctrl* (Windows) key while moving the mouse. All position changes must be confirmed in the edit panel to avoid unintentional changes in the measured trajectories and postures (Figure 22).

3.10 Insert Stimuli

You can add different stimuli in the *results viewer* (Figure 23). A right click inside the results viewer image (in the image tab) allows you to select one of the following stimulus marker:

- Point which is (x,y) position in the image
- Line which is a 1D line in the image
- Rectangle which is an arbitrary sized 2D rectangle in the image.
- Ellipsoid which is an arbitrary sized 2D ellipsoid in the image.

All above mentioned stimulus markers can be combined.

For a point, only the distance and bearing direction towards this point can be calculated (bearing angle definition: see below). If the stimulus is given by a line, the distance to this line is the minimal distance

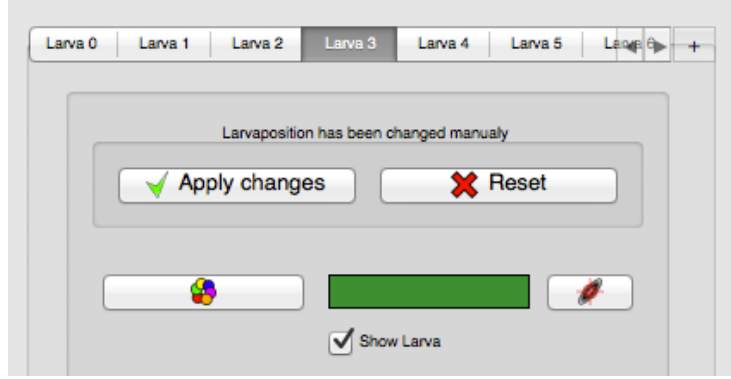


Figure 22: Dialog to confirm manual posture and trajectory changes.

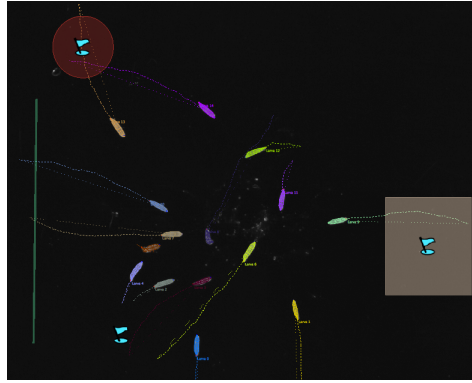


Figure 23: Several stimulus marker are placed in the results viewer image.

between the larval center of mass and the line. The bearing angle towards a line is the angle between the movement direction vector and the mid-point of the line. For 2D marker the distance to the centre of the marker, as well as the bearing direction is calculated. In addition, a binary indicator is stored to highlight time points in which the animal is inside this region. The size of all stimulus marker can be adjusted equivalent to the region of interest (see above).

3.11 Save Adjusted Results And Stimuli

To save the adjustments permanently, click on the *Save Results...* button in the top right of the results viewer. You will be asked to give a name for the new yml, csv and image file.

4 Features Calculated By FIMTrack

Here is a list of all features calculated by FIMTrack. These parameters are stored in the *table.csv* and the *output.yml* file. In general, the parameters can be separated into primary, secondary and tertiary parameters.

4.1 Primary Features

The primary features are directly calculated from the animals contour.

- **mom x** and **mom y** specifies the current (x,y) position of the center of mass (momentum; in pixel coordinates).

- **head x** and **head y** specifies the position of the head (in pixel coordinates).
- **tail x** and **tail y** specifies the position of the tail (in pixel coordinates).
- **Several points** along the spine (user defined number; in pixel coordinates).
- **Radii** along the inner spine points (in pixel).
- **area** specifies the covered area (in pixel).
- **perimeter** specifies the perimeter of the contour (in pixel).

4.2 Secondary Features

The secondary features are calculated from the primary features.

- **bodyBending** specifies the body bending. Bending of 180° means no bending, values > 180 indicate bending to the left and values < 180 specify bending to the right (Figure 24b; in degree).
- **spineLength** specifies the length of the spine (in pixel)
- **isLeftBended** and **isRightBended** is 1 if the larva is bended to the left or to the right respectively (this value is dependent on the preference option *Angle Threshold*; binary indicator).
- **isCoiled** is 1 if the larva is in a coiled body posture (i.e. head is touching the tail; binary indicator).
- **Distance to stimulus** is the distance to a given stimulus (in pixel).
- **In Stimulus Indicator** is true if the animal is inside a stimulus region (binary indicator).

4.3 Tertiary Features

The tertiary features are calculated from the primary features and over time.

- **movDirection** specifies the movement direction measured to the y-axes in degree. 90° is movement to the right, 270° is movement to the left (Figure 24a; in degree).
- **momDist** specifies the distance between two consecutive center of mass (i.e. momentum) positions (in pixels).
- **Velocity** is the velocity of the larva (in $\frac{mm}{s}$)
- **Acceleration** is given in $\frac{mm}{s^2}$.
- **accDist** specifies the accumulated distance over time (in pixel).
- **dstToOrigin** specifies the distance to the origin of the trajectories (in pixel).
- The bearing direction specifies movement direction towards or away from a stimulus: It is 0° if the animal moves straight towards as stimulus and 180° if the animals moves in the opposite direction (in degree).
- The peristalsis is indirectly given by the area over time (in pixel).
- **isGoPhase** is 1 if the larva is in a go phase (0 indicates larvae in stop phases; binary indicator).

5 Contact

For more informations contact b.risse@wwu.de (Benjamin Risse).

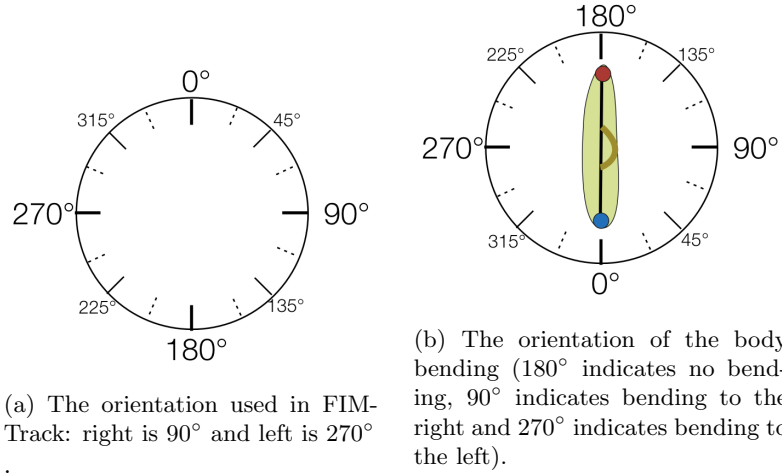


Figure 24: Orientations used the in FIMTrack parameters.

References

- [1] Benjamin Risse, Xiaoyi Jiang, and Christian Klämbt. FIM: Frustrated Total Internal Reflection Based Imaging for Biomedical Applications. *ERCIM News*, 95(Image Understanding):11–12, October 2013.
- [2] Benjamin Risse, Silke Thomas, Nils Otto, Tim Löpmeier, Dimitar Valkov, Xiaoyi Jiang, and Christian Klämbt. FIM, a Novel FTIR-Based Imaging Method for High Throughput Locomotion Analysis. *PloS one*, 8(1):e53963, January 2013.