FIMTrack Manual

Benjamin Risse
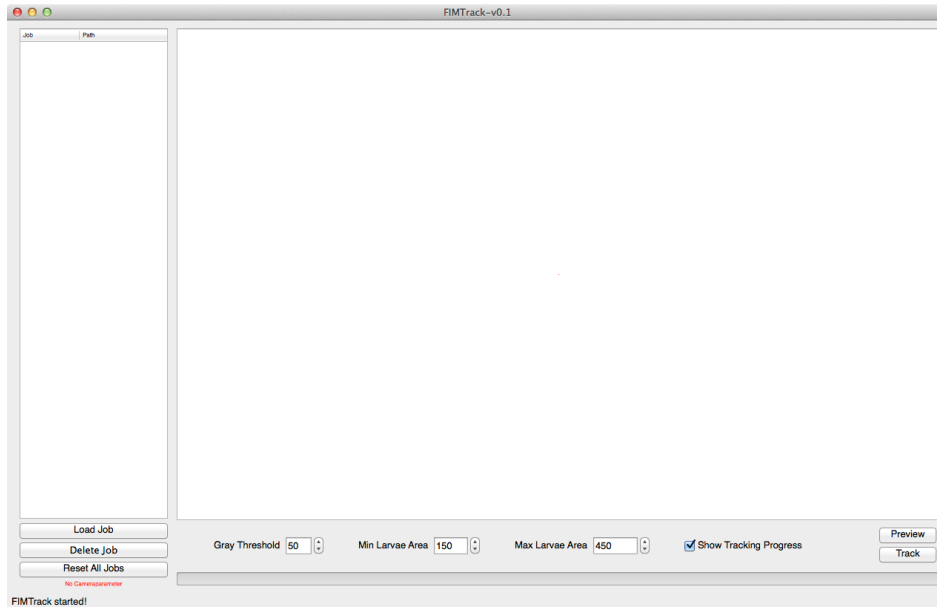
2014-04-10

Contents

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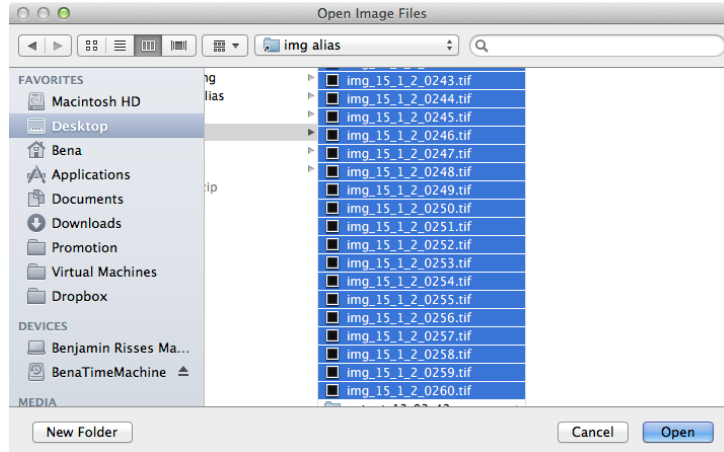
Figure 2: Select the images to be load into FIMTrack.

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 *str*) + *mouse wheel* or for faster zooming *cmd* (or *str*) + *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 an 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 5). 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 mm (unit $\frac{pixel}{mm}$) and the frames per second are given by the image acquisition rate of the camera.

3.3   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**
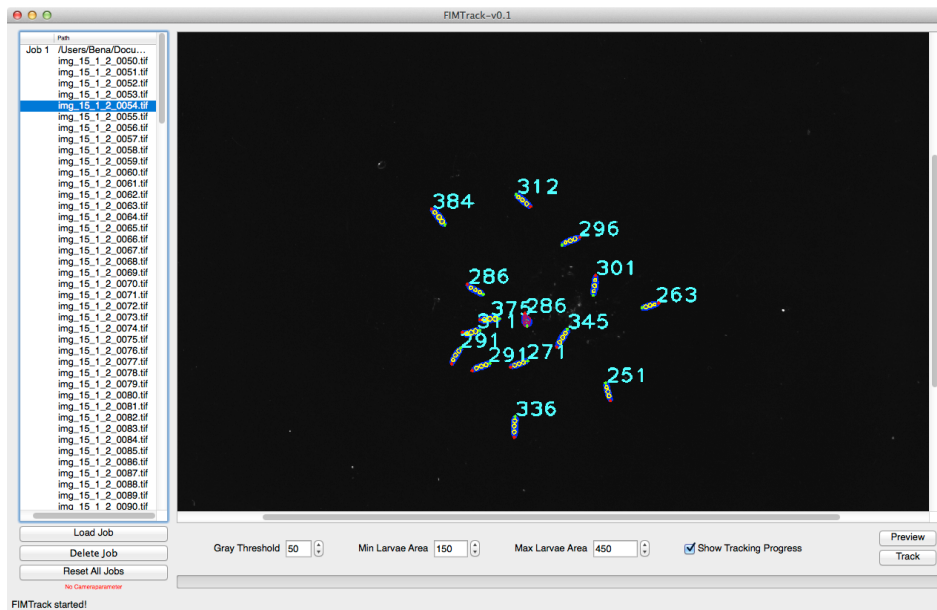
Figure 3: Preview image.

(a) Correct detected animal and its covered area.

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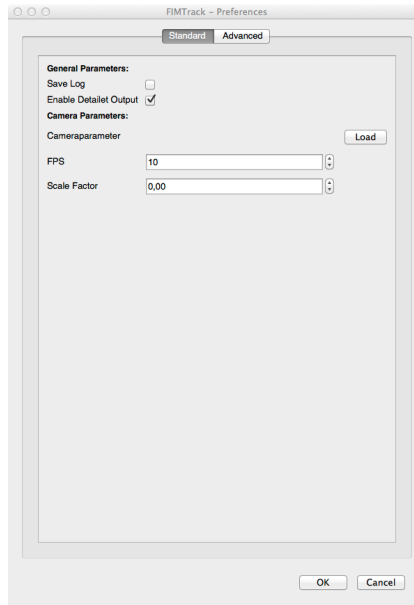

(c) Coiled larva (i.e. head is touching the tail).

4

Figure 5: Standard preferences dialog.

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.

3.4 How To Adjust 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 5).

- **Advanced** preferences include all tracking and feature extraction related parameters (Figure 6).

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.

- **Tracking Parameters** for general tracking

  - **Maximal Momentum Distance** specifies the maximal distance (in pixels) between two consecutive larval momentums (i.e. in two consecutive frames) so that they can still be assigned to the same larvae. If the larvae have a huge area or move very fast, this value needs to be incremented (if the larval ID changes between two frames, this value might be the problem).
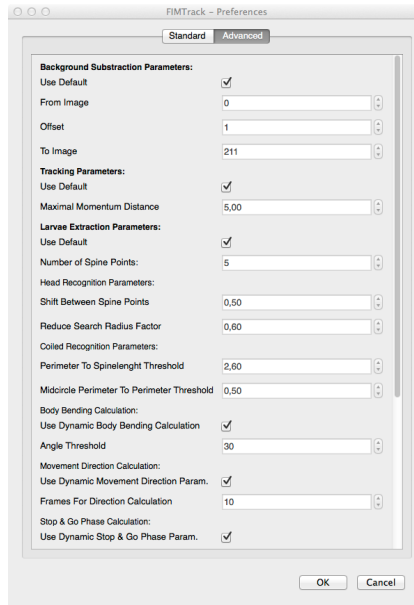
Figure 6: Advanced preferences dialog.

- **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.
  - **Head Recognition Parameters** to detect the head of the animals over time.
    * **Shift Between Spine Points**
    * **Reduce Search Radius Factor**
  - **Coiled Recognition Parameters** to detect coiled structures.
    * **Perimeter To Spinelength Threshold**
    * **Midcircle Perimeter To Perimeter Threshold**
  - **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 to short. Thus the direction is calculated for every frame by comparing the current frame i and the frame at time $i+offset$, whereas this parameters specifies $offset$.
  - **Stop & Go Phase Calculation** to classify stop and go phases
    * **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 to 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.

6

Figure 7: Several jobs are loaded for batch processing.

* **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.
    * **Minimal Triangle Side Length**
    * **Maximal Triangle Side Length**
    * **Maximal Curvature Points Distance**
    * **Curvature Window Size**

3.5 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 7). Single jobs can be removed from the list using the *Delete Job* button. *Reset All Jobs* removes all jobs from the list.

3.6 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.cvs** excel sheets containing all calculated features.

- **output.yml** YAML files containing all features and additional tracking informations (used in FIM-Track 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 bee seen in these images.

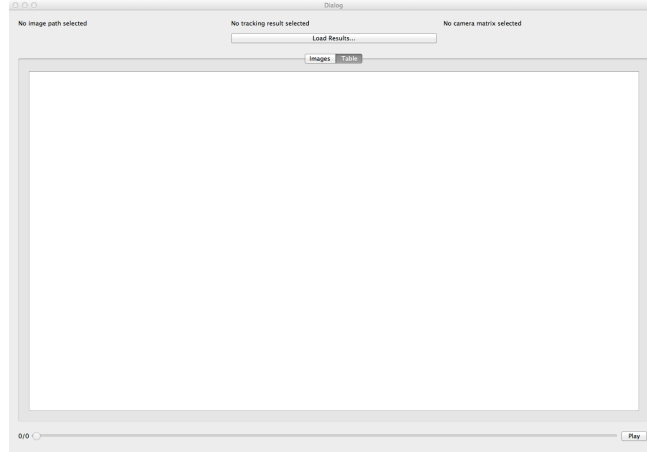- **tracksNoNumbers.tif** similar to tracks.tif but without larvae IDs

7

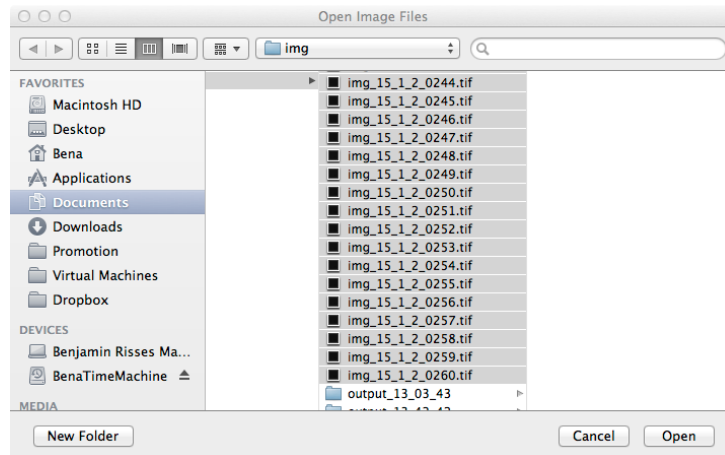Figure 8: Results viewer GUI dialog.

Figure 9: Select the same images used for tracking

3.7 View Results In FIMTrack

FIMTrack offers the possibility to review the calculated tracking results. Select *Edit > Results viewer...* to open the Results Viewer dialog (Figure 8). 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 9). Then you are asked to select the calculated tracking results; i.e. the *output.yml* file (Figure 10).

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 11). 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 12). 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 13).
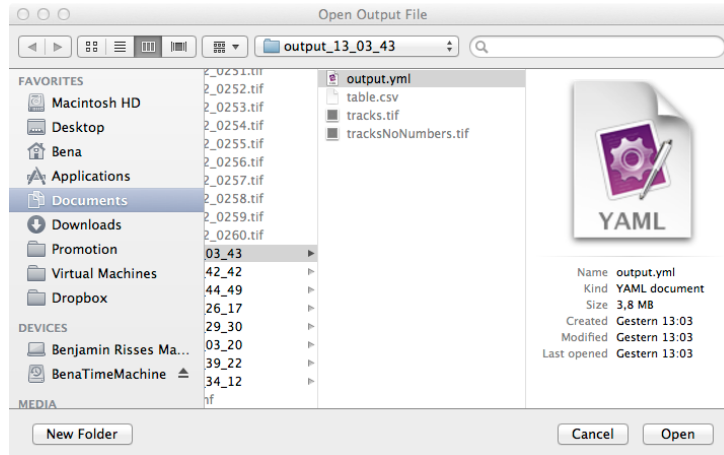
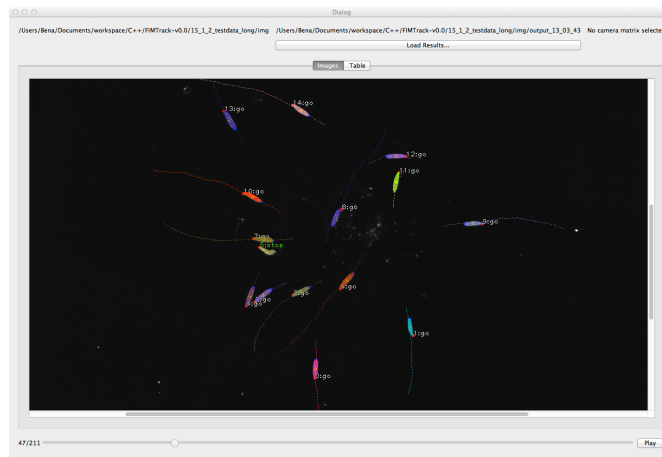Figure 10: Select the calculated *output.yml* file.

Figure 11: Image with plotted tracking results.

Figure 12: Current calculated features.

Figure 13: Current calculated features.

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.

- **area** specifies the covered area in pixel

- **mom x** and **mom y** specifies the current (x,y) position of the momentum

- **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.

- **isCoiled** is 1 if the larva is in a coiled body posture (i.e. head is touching the tail).

- **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*).

- **isGoPhase** is 1 if the larva is in a go phase (0 indicates larvae in stop phases).

- **movDirection** specifies the movement direction measured to the y-axes in degree. 90° is movement to the right, 270° is movement to the left.

- **spineLength** specifies the length of the spine in pixel

- **perimeter** specifies the perimeter of the contour in pixel.

- **momDist** specifies the distance between two consecutive momentum positions (i.e. the speed).

- **accDist** specifies the accumulated distance over time.

- **dstToOrigin** specifies the distance to the origin of the trajectories.

- **head x** and **head y** specifies the position of the head.

- **mid x** and **mid y** specifies the position of the mid spine point (sometimes different from the momentum).

- **tail x** and **tail y** specifies the position of the tail.

5   Contact

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

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.