# FIMTrack 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 <u>F</u>TIR-based Imaging <u>M</u>ethod, 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).

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Applications	Þ	img_15_1_2_0248.tif	
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Ownloads		■ img_15_1_2_0250.tif ■ img_15_1_2_0251.tif	
Promotion		img_15_1_2_0252.tif	
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🚞 Dropbox		img_15_1_2_0255.tif	
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MEDIA		img_15_1_2_0260.tif	
New Folder			Cancel Open

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).

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Save Log Enable Detailet Output Camera Parameters:	□ ∀	
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		OK Cancel

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).

Background Substraction Parameters:		
Use Default	✓	
From Image	0	
Offset	1	
To Image	211	¢.
Tracking Parameters:		
Use Default	$\checkmark$	
Maximal Momentum Distance	5,00	÷
Larvae Extraction Parameters:		
Use Default	✓	
Number of Spine Points:	5	-
Head Recognition Parameters:		
Shift Between Spine Points	0,50	÷.
Reduce Search Radius Factor	0,60	
Coiled Recognition Parameters:		
Perimeter To Spinelenght Threshold	2,60	÷.
Midcircle Perimeter To Perimeter Threshold	0,50	
Body Bending Calculation:		
Use Dynamic Body Bending Calculation	$\checkmark$	
Angle Threshold	30	
Movement Direction Calculation:		
Use Dynamic Movement Direction Param.	✓	
Frames For Direction Calculation	10	-
Stop & Go Phase Calculation:		
Use Dynamic Stop & Go Phase Param.	$\checkmark$	

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.



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 be seen in these images.
- tracksNoNumbers.tif similar to tracks.tif but without larvae IDs





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i bena	■ Img_15_1_2_0248.tif	
Applications	mg_15_1_2_0249.tif	
🕅 Documents	img_15_1_2_0250.tif	
	img_15_1_2_0251.tif	
U Downloads	img_15_1_2_0252.tif	
Promotion	img_15_1_2_0253.tif	
Virtual Machines	img_15_1_2_0254.tif	
	mg_15_1_2_0255.tif	
Dropbox	img_15_1_2_0256.tif	
DEVICES	img_15_1_2_0257.tif	
	img_15_1_2_0258.tif	
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	output_13_03_43	
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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).

000		Open Output File	
	😳 🔻 🚺 outp	ut_13_03_43 ‡	
FAVORITES Macintosh HD Desktop Bena Applications Documents Downloads Promotion Virtual Machines Dropbox DEVICES Benjamin Risses Ma Benjamin Risses Ma	2_0252.tif 2_0252.tif 2_0253.tif 2_0253.tif 2_0254.tif 2_0255.tif 2_0255.tif 2_0258.tif 2_0258.tif 2_0258.tif 2_0259.tif 2_0250.tif	output.yml table.csv tracks.tif tracks.NoNumbers.tif	Name outputyml Kind YAML document Size 3,8 M8 Created Cestern 13:03 Modified Cestern 13:03 Last opened Cestern 13:03
New Folder			Cancel Open





Figure 11: Image with plotted tracking results.

								Load Resu	lts				
Images Table													
	larva(0)	larva(1)	larva(2)	larva(3)	larva(4)	larva(5)	larva(6)	larva(7)	larva(4)	larva(9)	larva(10)	larva(11)	lan
rea:	286.5	220.5	285.5	243.5	308	261	335.5	330	244	259	280	285	287.5
nomusi	933	1128	869	945	813	844	1036	885	956	1211	858	1101	1048
nom_y:	1337	1267	1215	1211	1190	1145	1152	1118	1124	1092	1062	1050	955
odytending:	182.862	182.284	185.481	181.081	194.572	187.815	190.99	185.711	144.851	179.288	177.432	178.244	171.5
sColled:	0	0	0	0	0	0	0	0	0	0	0	0	0
sleftBendedt	0	0	0	0	0	0	0	0	0	0	0	0	0
skightBended:	0	0	0	0	0	0	0	0	1	0	0	0	0
sCoPhase:	1	1	1	1	1	1	1	1	0	1	1	1	1
novOirection	180	171.87	250.346	253.301	206.565	255.964	211.608	261.254	14.0362	72.8973	302.735	180	73.30
pinelength:	40.099	36.2499	40.2108	35.9251	42.6722	33.3124	43.0501	40.198	32.7817	36.0907	39.1668	41.5891	42.97
erimeter:	89.2132	83.6985	92.1838	85.0122	95.397	79.9411	96.8112	91.4558	77.1127	84.2843	90.5685	93.4558	96.66
nomDist:	0	0	0	0	0	0	0	0	0	0	0	0	0
ccDist.	0	0	0	0	0	0	0	0	0	0	0	0	0
IntToOrigin:	0	0	0	0	0	0	0	0	0	0	0	0	0
larToOrigin	0	0	0	0	0	0	0	0	0	0	0	0	0

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  $\mathbf{x}$  and mid  $\mathbf{y}$  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

- 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.