GershowLab

Larva Track Extractor

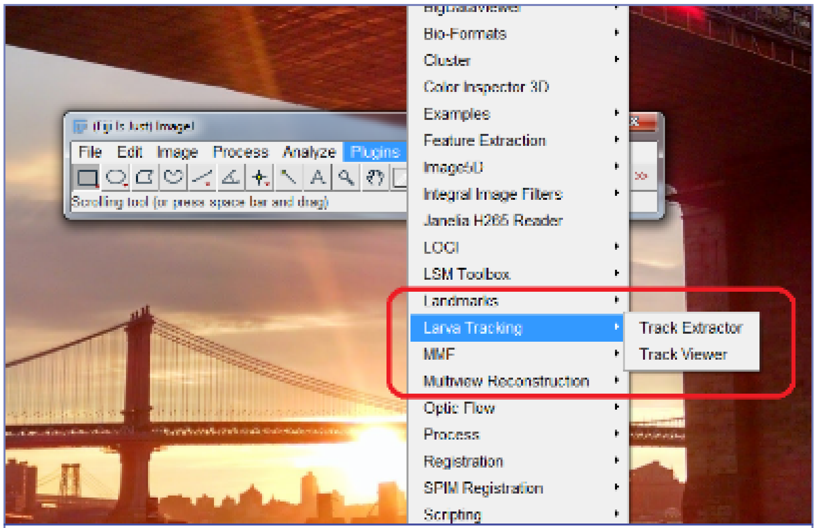
Overview

This plugin provides the tools to extract tracks and postural data from videos with multiple crawling larvae.

Installation

Download the Larva_Track_Extractor-1.0.0-SNAPSHOT.jar file from https://github.com/NBernat/Track-Extraction-Java/releases. Place the jar file in the plugins directory of your Fiji directory (e.g. C:\Fiji.app\plugins).

When you restart Fiji, there will be a new menu item in the "Plugins" menu called "Larva Tracking". This has three plugins: *Extract Tracks from Current Window*, the *Track Extractor*, and the *Track Viewer*. (note picture shows older version)



The location of the plugins after installation

Track Extractor Operation

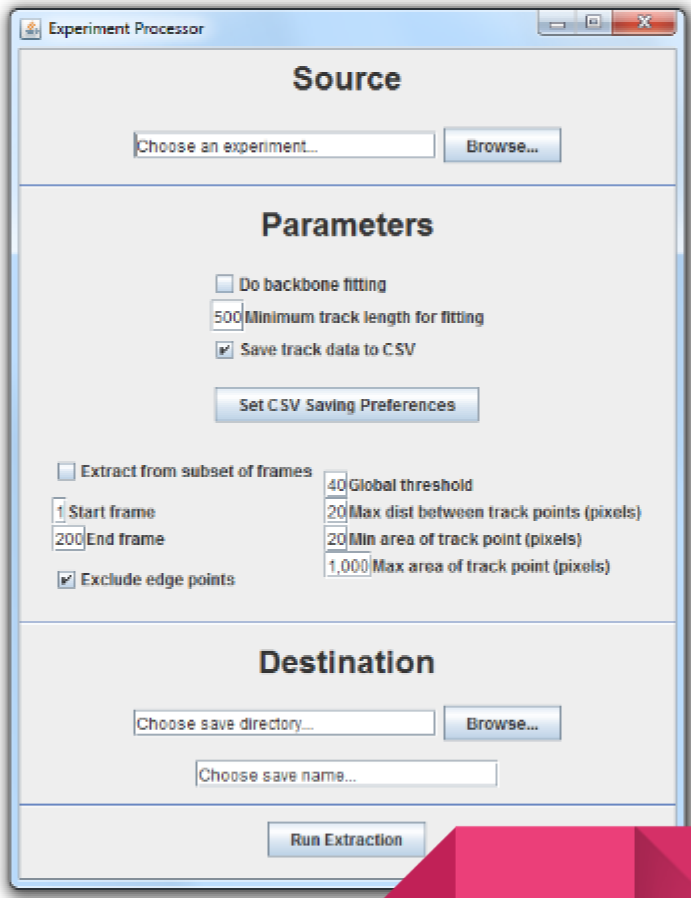
Source & Destination:

To use the current windows as a source, use the Extract Tracks from Current Window plugin. Otherwise, use the Track Extractor Plugin and under Source choose the name of your video file. Any video file that can be opened with ImageJ's "File>Open..." function can be opened by entering the file name. Note: If the video has to be opened through the "File>Import" function, open the file through Fiji, and use the extract tracks from current window plugin.

Under Destination, enter the directory where the extracted tracks will be saved, as well as the name of the output files.

Parameters:

- Do backbone fitting: Runs advanced software to produce improved identification of the midline coordinates that represent the larva's posture.
- Minimum track length for fitting: If "Do backbone fitting" is selected, this is used to filter out erroneous short tracks, reducing the time-consuming process of trying to identify a spine
- Save track data to CSV: Generates a CSV file from the track data



☐ Do backbone fitting
500 Minimum track length for fitting
☑ Save track data to CSV
☐ Extract from subset of frames
1 Start frame
200 End frame
☑ Exclude edge points
40 Global threshold
20 Max dist between track points (pixels)
20 Min area of track point (pixels)
1,000 Max area of track point (pixels)

The Experiment Processor Win

- Set CSV Saving Preferences: Opens a window to select which values for each trackpoint should be written to the CSV

- Extract from subset of frames, Start frame, & End frame: If "Extract from subset" is selected, the extractor only extracts tracks from the frames specified in the start frame and end frame fields
- Exclude edge points: If selected, the extractor ignores any points touching the border of the image

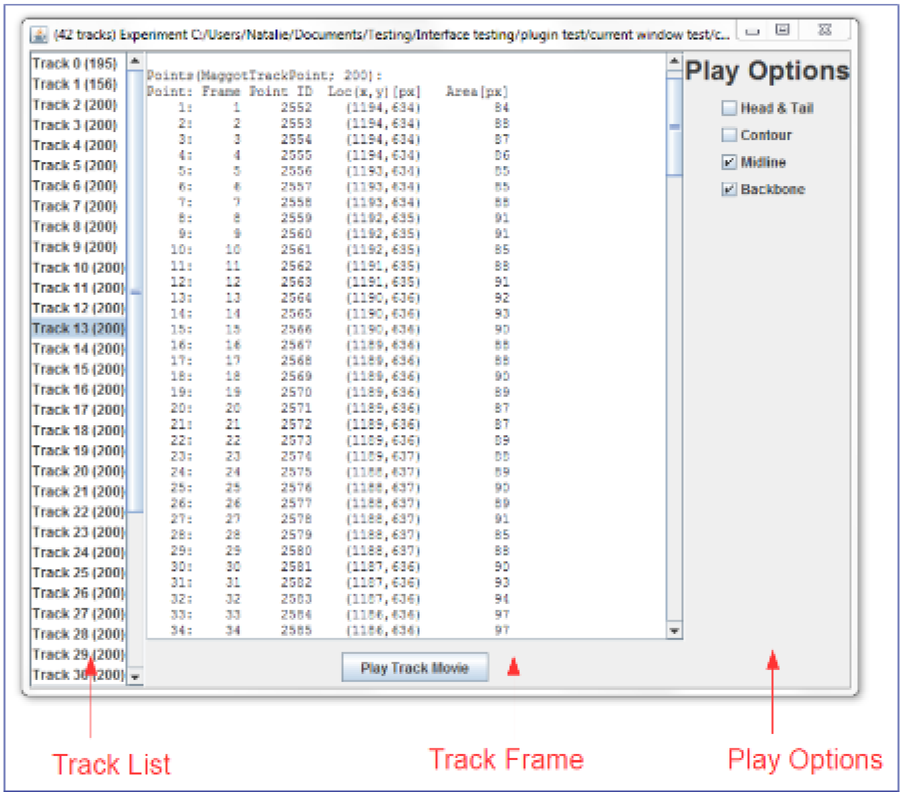
- Global threshold: Any pixels with a grayscale intensity (0-255) below the global threshold will be ignored when finding larva (See **Tips** below)
- Max dist between track points: the maximum distance (in pixels) which a point is allowed to travel between frames in order to be considered part of the same track
- Min & Max area of track point: The minimum and maximum area (in pixels) used to identify a particle as a larva (See **Tips** below)

Running:

The "Run Extraction" button will open the specified Source video, extract tracks according to the specified parameters, and save the track data to a .prejav file. If "Do backbone fitting" is selected, the tracks with newly identified postural data will be saved into a .jav file. (The .prejav and .jav files can be viewed through the Track Viewer plugin). If "Save track data to CSV" is selected, the tracks will be written to a .csv file. All output files will be located in the specified destination directory, and will have the specified file name. If files with the same name already exist, they will be overwritten. Note -- it may look like nothing is happening; you can check for a file called “ProcessingLog.txt” in your output directory to confirm that the plugin is running.

Track Viewer Operation

The Track Viewer prompts the user to choose a .jav or .prejav file, and opens it in the experiment view.



(42 tracks) Experiment C:/Users/Natalie/Documents/Testing/Interface testing/plugin test/current window test/c...

Track 0 (195)
Track 1 (156)
Track 2 (200)
Track 3 (200)
Track 4 (200)
Track 5 (200)
Track 6 (200)
Track 7 (200)
Track 8 (200)
Track 9 (200)
Track 10 (200)
Track 11 (200)
Track 12 (200)
Track 13 (200)
Track 14 (200)
Track 15 (200)
Track 16 (200)
Track 17 (200)
Track 18 (200)
Track 19 (200)
Track 20 (200)
Track 21 (200)
Track 22 (200)
Track 23 (200)
Track 24 (200)
Track 25 (200)
Track 26 (200)
Track 27 (200)
Track 28 (200)
Track 29 (200)
Track 30 (200)

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Points(MaggotTrackPoint; 200):
Point: Frame Point ID  Loc(x,y)[px]    Area[px]
    1:     1     2552    (1194,634)          84
    2:     2     2553    (1194,634)          88
    3:     3     2554    (1194,634)          87
    4:     4     2555    (1194,634)          86
    5:     5     2556    (1193,634)          85
    6:     6     2557    (1193,634)          85
    7:     7     2558    (1193,634)          88
    8:     8     2559    (1192,635)          91
    9:     9     2560    (1192,635)          91
   10:    10     2561    (1192,635)          85
   11:    11     2562    (1191,635)          88
   12:    12     2563    (1191,635)          91
   13:    13     2564    (1190,636)          92
   14:    14     2565    (1190,636)          93
   15:    15     2566    (1190,636)          90
   16:    16     2567    (1189,636)          88
   17:    17     2568    (1189,636)          88
   18:    18     2569    (1189,636)          90
   19:    19     2570    (1189,636)          89
   20:    20     2571    (1189,636)          87
   21:    21     2572    (1189,636)          87
   22:    22     2573    (1189,636)          89
   23:    23     2574    (1189,637)          88
   24:    24     2575    (1188,637)          89
   25:    25     2576    (1188,637)          90
   26:    26     2577    (1188,637)          89
   27:    27     2578    (1188,637)          91
   28:    28     2579    (1188,637)          85
   29:    29     2580    (1188,637)          88
   30:    30     2581    (1187,636)          90
   31:    31     2582    (1187,636)          93
   32:    32     2583    (1187,636)          94
   33:    33     2584    (1186,636)          97
   34:    34     2585    (1186,636)          97
```

Play Options

☐ Head & Tail
☐ Contour
☑ Midline
☑ Backbone

The Track Viewer window

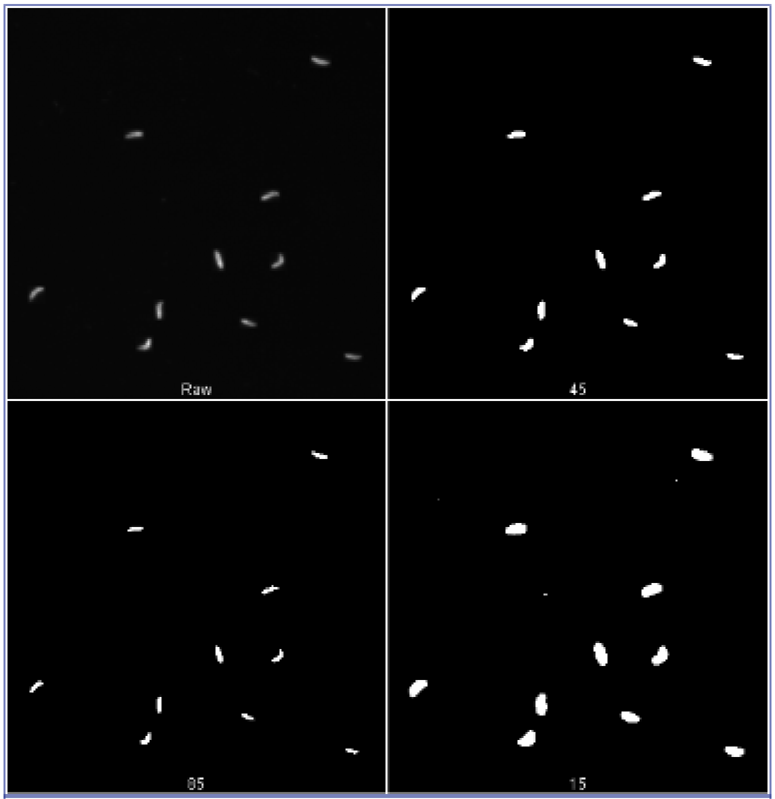
(a) Track List: Each track is listed with the title "Track [Track ID] ([# of points])". Selecting a track will load the track into the track frame. Double-clicking on a track will open a window with the track movie.

(b) Track Frame: Information about the selected track is displayed, including a summary of the track and a summary of each point in the track.

(c) Play options: Whichever features are selected will be drawn on each frame of the larva movie.

(d) Two other buttons allow you to export the selected track to a CSV files or to a separate experiment file.

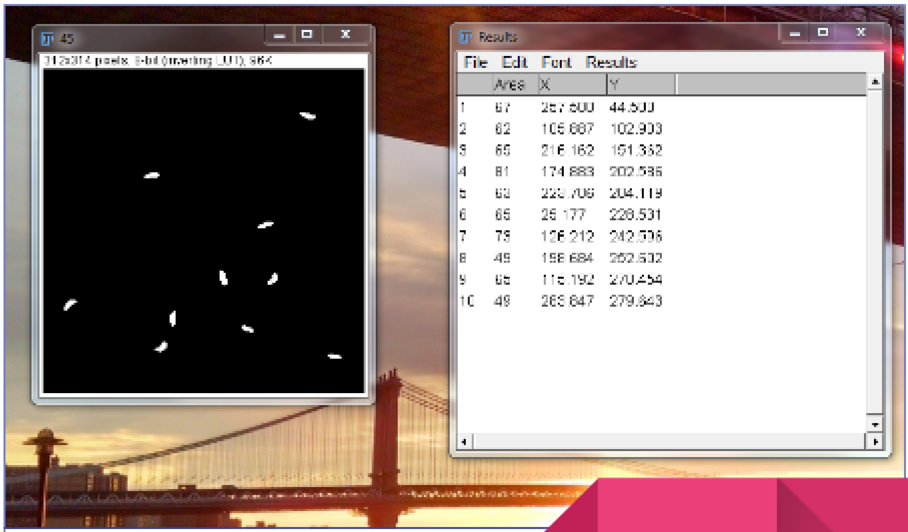
Tips



An image clip with various thresholds, labeled on image. (UL) The raw image data. (UR) a reasonable threshold value. (BL) A relatively high threshold value; pixels are eroded. (BR) A rather low threshold value; unnecessary pixels are included in analysis.

1) To find the **threshold** value for your video, open the video through Fiji. Open the B&W thresholder (Ctrl+Shift+T, or Image> Adjust> Threshold). Manually set the upper threshold value to 255, and set the lower threshold to a reasonable value (see examples at right). Note: a clean image with little dirt and proper lighting will have a large window of threshold values that will produce reasonable results.

2) To determine the **min area and max area of track point**, take the thresholded image and perform Particle Analysis (Analyze> Analyze Particles) with an area range of [0-Infinity]. Look at the distribution of area



	Area	X	Y
1	67	257.500	44.500
2	62	105.887	102.903
3	65	216.162	151.362
4	81	174.883	202.536
5	63	223.706	204.119
6	65	25.177	228.531
7	73	126.212	242.596
8	49	158.684	252.602
9	65	116.192	270.454
10	49	263.847	279.843

Particle analysis results. Min and max partic
reasonable choice of min and max area f

values given in the results table, and choose a range that includes all possible (relevant) particles.