Arena Worm Tracker

Overview:
We developed a microfluidics-based system for the analysis of *C. elegans* locomotory behavior in response to defined spatial and temporal stimulus patterns. We designed 2 x 2 cm structured arenas with regulated fluid flow that allow *C. elegans* to perform crawling-like locomotion resembling normal behavior on agar surfaces. Stimuli are delivered in the fluid phase and odor-evoked behaviors in the arena are automatically captured, segmented and analyzed using the MATLAB scripts available here.

Any updates to this software will be available at: http://arenawormtracker.dyndns.org

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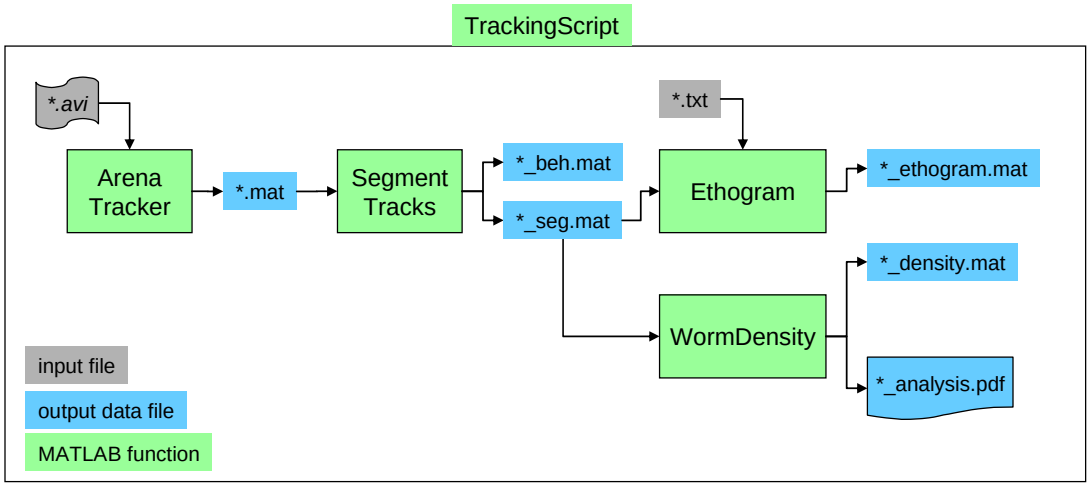
Installation Instructions: (assumes familiarity with MATLAB environment)

1. Unzip and extract MATLAB library folder ("ArenaWormTracker") to an appropriate location.
2. Set MATLAB path (File → Set Path… → Add folder with Subfolders…) to the chosen library location.

MATLAB function and data file notes:

Analysis flowchart:



MATLAB scripts (m-files) accomplish the following basic tasks:

TrackingScript: calls the following 4 functions in sequence:

1. **ArenaTracker**: Segment video file to obtain worm tracks. Code adapted from the parallel worm tracker (http://wormsense.stanford.edu/tracker/).
2. **SegmentTracks**: Identifies instantaneous behavioral states from worm tracks and morphological data.
3. **Ethogram**: Displays ethogram and summarizes behavioral state probability and speed over time. Allows time-shifting to align stimulus pulses.
4. **WormDensity**: Summarizes behavioral state and speed data over space and time.

Video tracking, behavior segmentation, and data analysis were performed using MATLAB v7.0.1 with the Image Processing and Statistics toolboxes. Scripts have been tested on Windows XP and 7.

Input files:

1. Video file (*.avi)
   - uncompressed or compressed with MATLAB-compatible codec, e.g. Indeo 5

2. Experiment information file (*.txt) - *optional*
   - contains information about the experiment. Especially useful for pulse assays to specify the pulse timing and to automatically determine flow properties.

Pulse assay example:

```
Genotype,  N2
Stimulus,  1,4,IAA,0.92
         ,  7,7.5,IAA,0.92
         ,  8,8.5,IAA,0.92
         ,  11,12,IAA,0.92
         ,  13,14,IAA,0.92
NumCycles, 4
CycleLen,  15
FlowVel,   0.88
Delay,     3.53
```

- specifies four 15 min cycles with 0.92µM IAA stimulus from 1-4 min, 7-7.5 min, etc.
- flow velocity = 0.88 mm/s
- time delay between valve switch and stimulus switch upstream of device = 3.53 s

Stripe assay example:

```
Genotype,  N2
Stimulus,  0,80,IAA,0,0.92,0,1.84,0
         ,  80,120,IAA,0
```

- specifies five stripes from 0-80 min, containing from top: 0 µM, 0.92µM, 0 µM, 1.84µM, 0 µM IAA and 0 µM IAA throughout from 80-120 min

Output data files:

Gray indicates variables duplicated in previous files. Numbers preceding a period define column numbers. Numbers preceding a colon indicate the meaning of a data or index value.

1. Tracks file (*.mat): *Worm position and morphology only*

AllData	Data matrix containing tracking information about every thresholded object, regardless of size	
	1. Frame #	
	2. Object size (pix^2)	
	3-4. Centroid X-Y	
	5. Object eccentricity	
DyeData	Data matrix for analysis of dye patterns	
ExpData	Experiment data	
File	Filenames from ArenaTracker	
Tracks.___	Track structure	
Path	x-y position matrix of centroid (pix)	
Frames	vector of frame numbers	
Size	vector of object size (pix^2)	
Eccentricity	vector of eccentricity	
MajorAxes	vector of major axis (pix)	
MinorAxes	vector of minor axis (pix)	
Orientation	vector of orientation (deg)	
Box	x-y position matrix of bounding box	
Frame.Image	binary image of thresholded object	
background	background image	

2. Segmented tracks file (*_seg.mat): *Tracks file with worm behavior*

AllData	Data matrix containing tracking information about every thresholded object, regardless of size	
		1. Frame #
		2. Object size (pix^2)
		3-4. Centroid X-Y
		5. Object eccentricity
DyeData	Data matrix for analysis of dye patterns	
ExpData	Experiment data	
File	Filenames from ArenaTracker	
SegSettings	Segmentation settings	
Tracks.___	Track structure	
Path	x-y position matrix of centroid (pix)	
Frames	vector of frame numbers	
Size	vector of object size (pix^2)	
Eccentricity	vector of eccentricity	
MajorAxes	vector of major axis (pix)	
MinorAxes	vector of minor axis (pix)	
Orientation	vector of orientation (deg)	
Box	x-y position matrix of bounding box	
Frame.Image	binary image of thresholded object	
Code	segment code (1-10)	
Distance	vector of distance traveled since previous frame (pix)	
Speed	vector of speed (mm/s)	
PathAngle	vector of path angle (deg)	
PathAngVel	vector of path angular velocity (deg/fr)	
Reverse	vector of reversing frames	
OriginalDistance	vector of distance traveled, before pause analysis	
Segment	structure of segment analysis	
Stall	vector of pausing frames	
NoStall	vector of non-pausing frames	
Beh	behavior code	
		1: Forward straight
		2: Forward curve
		3: Reverse
		4: Pause
		5: Pirouette Reverse
		6: Pirouette Forward
		7: Unknown
		8: Out of bounds
OriginalTrack	original track linked to current track, aka animal #	
background	background image	
trackstats	Tracking statistics	
		1. Length of track (frames)
		2. Animal #

3. Behavior tracks file (*_beh.mat): *Condensed version of Segmented tracks file*

Tracks.___	Track structure	
Frames	vector of frame numbers	
Speed	vector of speed (mm/s)	
PathAngle	vector of path angle (deg)	
Beh	behavior code	
		1: Forward straight
		2: Forward curve
		3: Reverse
		4: Pause
		5: Pirouette Reverse
		6: Pirouette Forward
		7: Unknown
		8: Out of bounds
OriginalTrack	original track linked to current track, aka animal #	
X, Y	x- and y-position of centroid	

4. Ethogram file (*_ethogram.mat): *Worm behavior, time-shifted for pulse assay*

ExpData	Experiment data
Data.___	Data structure containing matrices (row = animal #; col = timepoint) of:
xmat	x-position (pix)
ymat	y-position (pix)
dirmat	direction (deg)
behmat	behavior code
	1: Forward straight
	2: Forward curve
	3: Reverse
	4: Pause
	5: Pirouette Reverse
	6: Pirouette Forward
	7: Unknown
	8: Out of bounds
spdmat	Speed (mm/s), negative values = reverse.
behprob	matrix of instantaneous behvaior probability (behavior codes 1-6 only)
behnum	number of animal-frames ber time point (useful for weighted averages)
Data.speed.___	Average instantaneous speed for:
.all	all behaviors
.fwdpause	forward and pause behaviors
.forward	forward runs only

5. WormDensity file (*_density.mat): *Worm behavior, averaged over time and space*

All.___	# animal-frames per bin of All animals, including out of bounds and collisions. For collisions, number of frames equals estimated number of animals in the collision.
Track.___	# animal-frames per bin of all valid tracked animals, excluding out-of-bounds, collisions, and unknown behaviors.
Beh(1:6).___	# animal-frames per bin of animals in a specific behavior:
	1: Forward Run
	2: Pause
	3: Reversal (non-omega)
	4: Pirouette Reverse
	5: Pirouette Forward
	6: Pirouette Rev or Fwd
Speed.FP.___	Average speed (mm/s) per bin for animals in a forward run or pausing.
.wt.___	Weight per bin, i.e. number of animal-frames contributing to each speed average per bin
XYTimeD	4-D matrix, Xbin, Ybin, Timebin, Direction (1=up, 2=horiz, 3=down)
XYTime	3-D matrix, sum of XYTimeD over all D
XY	2-D ...
TimeX	2-D
TimeY	2-D
XYD	3-D
XTimeD	3-D
YTimeD	3-D
Time	1-D vector of time bins (mins)
X	1-D vector of x-position (mm)
Y	1-D vector of y-position (mm)
ExpData	Experiment data
File	Filenames from ArenaTracker
FileInfo	Additional filenames
SegData	Data matrix of all segments per experiment
	1. Track #
	2. Segment # (of Track)
	3. Behavior Code
	4. Behavior Run (Run = 1 vs. Turn = 0)
	5. Segment Length (frames)
SegPos	Position matrix of all segments per experiment
	1-3. SegmentStart (frame, X, Y of starting point)

	4-6. SegmentEnd (frame, X, Y of ending point) 7-8. SegmentCentroid (X, Y of centroid) 9-12. SegmentMinMax (minX, minY, maxX, maxY)
SegDir	Direction matrix of all segments per experiment 1. angUD (ending direction of segment: 1=up (-y); 2=horiz; 3=dn (+y) 2-3. SegmentDir (mean, std of all angles in segment: +x axis; ccw) 4. SegmentBoundHead (initial, final angle) 5-17. Histogram of angles (# frames in 0,30,60,90,... 330)
TrackData	Data matrix of every animal and time point during the experiment 1. Animal# 2. Time (min) 3-4. XY centroid position 5. Animal Behavior 1: out of bounds 2: forward run 3: pause 4: reversal 5: omega reverse 6: omega forward 6. Speed (forward & pause only, otherwise NaN) 7. Animal Direction Category -1: -y ("up") 0: horizontal 1: +y 2: no direction 8. Animal Direction angle (deg)
TurnData	Data matrix of every turn during the experiment 1. time(fr) 2. posX 3. posY 4. turnID 1: forward run (2= with reverse frames) 3: forward shift 4: 60deg curve 5: unknown short turning segment 6: long curve (non-pirouette) 7: pirouette 8: unknown long turning segment 5. priorDirUD 6. afterDirUD
segidx	segment # of forward run prior to turn for each row in TurnData