User Manual for the single worm tracking software package.

This is the user manual for the 1.0 version of the single worm tracking software package: worm_BBQ. This package can process single worm video and provide the location of the worm skeleton and angles along the worm's body. The software can deal with fast moving worms, coiling worms. The codes are written in matlab, and need –mex to run some of the functions. Please follow the instruction below to run the codes.

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This software package is for tracking a single worm from a video captured from a camera

If the original video contains multiple worms, the whole operation consists 3 steps (if a single worm video is ready at hand, please jump to the 2nd step directly).

- 1. Run 'Create_Test.m' to create the single worm test video. (around 20-60 seconds)

 The 'workingDir' and 'filename' should be changed accordingly to read the input video from the right path. After seeing a frame from the video, the user can crop part of the image to generate a single worm video with smaller size and shorter length. The cropped video will be save as a name as '['Sample_Video\Video\Video_', date, '.avi']'.
- 2. Run 'Generate_Frenet_1.m' to generate the skeleton data of the worm in the first frame image. (less than 5 seconds)

 The path 'addpath' function and the input video 'fname' should be uniform with the cropped video obtained in step 1. 'Generate_Frenet_1.m' will show the first frame of the video, its binary image, the curvature of the contour of the worm, and finally save the skeleton and Frenet dataset 'Frenet_Pt' into the '.mat' file shown in the last line of the code.
- 3. Run 'Main_TwoLayers.m' as the main function to track the single worm (approximately 2~3 seconds per frame, total time depends on the frame number of the video). The result is a tracking video with '.tif' format saved in the 'results' folder. 'addpat' is the local folder of 'Main_TwoLayers.m'. 'vr' is the input video same as 'fname' in 2nd step. We need to 'load' the '.mat' dataset saved in the 2nd step.

File parameters need to be changed accordingly:

Name:	Value
addpath current working folder	
filename	the file to save current tracking video
fname	the avi file to save current video
load '.mat'	load the dataset obtained previously for the first several frame of video

Model parameters can be changed according to the input video:

Name:	Value	Default
N_particles	number of particles (hypotheses) are saved after each iteration. It is	10
	also the number of hypotheses saved after 1 st layer and 2 nd layer.	
sub_num_1	number of sub-particles generated in the 1 st layer, should >=100	100
Sub_num_2	number of sub-particles generated in the 2 nd layer, should >=50	50
seg_len	the length (pixels) of each segment of the skeleton (this value should	8
	be equal to 'seg_len' in 'Generate_Frenet_1.m')	
Xstd_rgb	estimated variance of the image. Use large value if the variance of the	40~80
	image is large. This value relates to the probability calculated for each	
	hypotheses	
var_speed	estimated variance of the speed, in terms of pixels per frame	2~10
var_len	estimated variance of the length of the worm, utilized in the 1st layer,	10
	in terms of pixel per frame	
width	the half width of the worm (no. pixel = width * 2)	3 or 3.5
para_thre	the threshold parameter for segmenting the contour	0.7~1
len_max	the maximum length estimated	~100
len_min	the minimum length esimtated	~70
fps	video rate of the .avi file generated	10
tt	weighted average several best hypotheses to generate the estimated	3
	skeleton.	
ii	show best several hypotheses in the result video, from red to pink	5:-1:1

Eg. Parameters for some test videos:

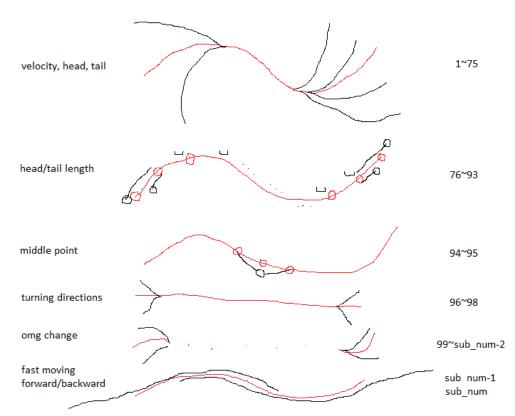
N_partiles: 10; sub_num_1 = 100; sub_num_2 = 50; seg_len = 8; var_len = 10;

load source file (.mat)	Frent_Coil	Frent_Pt_full	Frenet_1903	Frenet_0904
vr (.avi)	Video_coil	Video_coil_full	Video_Test1	Video_Test2
Xst_rgb	40	40	40	75
var_speed	5	5	4	4
var_len	10	10	10	10
len_max	105	105	85	85
len_min	85	85	70	70

Parameters Explanation in other functions

parameter name	in which function	Explanation	default
var_ske_direc	var_ske_direc Hypo_1st middle point variance along		0
		tangent direction	
var_direct	Hypo_1st	middle point random noise	0
var_mid	Hypo_1st	position variance of middle	0.25
		point	
para_1_2	Hypo_1st	probability of ordinary length	0.9
		change model, also means (1-	
		para_1_2) probability in double	
		length change model	
scenario	Hypo_2nd	no. of hypo for different	[4,5,7,2,12]
		scenario	
var_len_tail	Hypo_2nd	variance of change of tail	1
		segment	
var_len_head	Hypo_2nd	variance of change of head	1
		segment	
t, ts	calc_log_likelihood_	step points	about m_fre_pt
	Worm_1st/2nd		
ор	calc_log_likelihood_	overlap penalty, added in the D	20 or 25
	Worm_1st/2nd		
se	calc_log_likelihood_	'imclose' or 'imopen'	[0 1 0;1 1 1;0 1 0]
	Worm_1st/2nd	parameter, the area disk	
img_ratio	calc_log_likelihood_	estimated pixels in the worm	eg. ~700
	Worm_1st/2nd	body, in order to normalize D	
		making it close to 1. Do not	
		need to be accurate	
thre	ang2ang_1st,	the threshold/maximum	1.4
	ang2ang_2nd,	absolute angle change of two	
	ang2ang_1st_narro	adjacent segments on	
	W	skeleton.	
ang_head_chg	ang2ang_1st	angle change near the head	ang_head_i *
			[0.15,0.25,0.4,0.55]
ang_tail_chg	ang2ang_1st	angle change near the tail	ang_tail_i *
			[0.75,0.55,0.4,0.2]
distr	ang2ang_1st,	random angle change	exp(-0.5*(len_ang-
	ang2ang_1st_narro	magnitude distribution	(1:len_ang)))
,	W		
var_case{1,2,3,4,5}	ang2ang_2nd	variance of the change	var_case1 = 0.5;
		magnitudes for different	var_case2 = 0.2;
		scenarios/cases {1,2,3,4,5}	var_case3 = 0.3;
			var_case4 = 0.25;
			var_case5 = 0.2;
num_pt	ang2ang_2nd	number of points considered in	6
		case 1, in which 2 points near	
		tail, others near head	

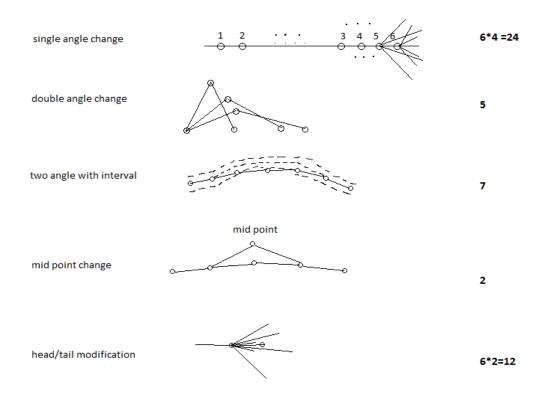
Hypotheses indexes corresponding to 'hypo_1st.m'



jj=1~100+	hypotheses meaning
1~75	They are the combination of velocity, head angle and tail angle: $jj = 1 \sim 75$
	(5*5*3 = 75). The locomotion represents obvious turning directions, but the
	length of worm stays the same.
	Velocities: -2, -1, 0 ,1, 2
	Head angles: -2, -1, 0, 1, 2
	Tail angles: -1, 0, 1
	eg. jj = 15 means velocity =-2, head angle = 2, tail angle = 1.
76~93	They are the combination of angles and length changes. (3*6=18)
	angles = -1,0,1 length changes = {head/tail/all, increase/decrease}
	jj = 76~81: angles = -1
	jj = 82~87: angles = 0
	jj = 88~93: angles = 1
	In each sub-slot, jjj = mod((jj-75),6)
	jjj = 1: head segment length increase jjj = 2: head segment length decrease
	jjj = 3: tail segment length increase jjj = 4: tail segment length decrease
	jjj = 5: all segment length increase jjj = 6: all segment length decrease
	In addition, for each jjj, there are 10% probability that a double length change
	are implemented in 2 segments near head/tail.

94~95	middle point shift (MPS)
	MPS should change the angle and middle point location accordingly. Here, the
	angle change is completed in function 'mid_pt_chg'. The location shift is
	completed by shifting a distance '[X_mid, Y_mid]'.
96~98	Turning direction dramatically. 50% probability head turns and 50% tail. The
	angle change is completed in the function 'ang2ang_1st_narrow'.
99~sub_num-2	omg randomly changes
sub_num -1	moving fast backwards, with length of 2 segments
sub_num	moving fast forwards, with length of 2 segments

Hypotheses indexes corresponding to 'hypo_2nd.m'



jj	hypotheses meaning
1~24	single angle change (6*4 = 24)
	6 points on skeleton in total, in which 4 points near head and 2 points near tail.
	for each point, 4 angle changes are generated randomly.
25~29	double adjacent angle change
	randomly choose 2 adjacent points on skeleton, and change their angles at the
	same time with one plus and the other minus,
30~36	two angles change with interval
	randomly choose 2 points on skeleton expect for points near head/tail, change
	their angles at the same time with one plus and the other minus
37~38	middle point change
	change the position of middle point, need to change the 2 adjacent angles at the
	same time
39~50	head/tail special modification