**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

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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\_',date,'.avi' ]’.

1. 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.

1. Run ‘Main\_TwoLayers.m’ as the main function to track the single worm (approximately 2~3 seconds per frame, total time depends on the number of frame in the video). The result is a tracking video of ‘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.

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Functions and Parameters in ‘Main\_TwoLayers.m’

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*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 1st frame of the 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 also the number of hypotheses saved after 1st layer and 2nd layer. | 10 |
| sub\_num\_1 | number of sub-particles generated in the 1st layer, should >=100 | 100 |
| Sub\_num\_2 | number of sub-particles generated in the 2nd layer, should >=50 | 50 |
| **seg\_len** | the length (pixels) of each segment of the skeleton (this value should be equal to ‘seg\_len’ in ‘Generate\_Frenet\_1.m’) | 8 |
| **Xstd\_rgb** | estimated variance of the image. Use large value if the variance of the image is large. This value relates to the probability calculated for each hypotheses | 40~80 |
| **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, in terms of pixel per frame | 10 |
| **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 |

*Eg. Parameters for some test videos:*

N\_partiles: 10; sub\_num\_1 = 100; sub\_num\_2 = 50; seg\_len = 8; var\_len = 10;

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| --- | --- | --- | --- | --- |
| 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 in other sub-functions:*

|  |  |  |  |
| --- | --- | --- | --- |
| function name | parameter name | Value | default |
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Hypotheses indexes corresponding to ‘hypo\_1st.m’

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| --- | --- |
| jj | hypotheses meaning |
| 1~75 |  |
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jj = 1 ~ 75 (5\*5\*3 = 75): combination of velocity, head angle and tail angle

Velocities: -2, -1, 0 ,1, 2

Head angles: -2, -1, 0, 1, 2

Tail angles: -1, 0, 1

2nd layer:

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| --- | --- |
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