

User's Manual

ver. 3.0

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1. Before Using ChaIN

Download ChaIN (zip file) at: http://www.bio.nagoya-u.ac.jp/~NC_home/chain_E.html

Keep ChaIN.exe and ChaIN.ini in the same folder

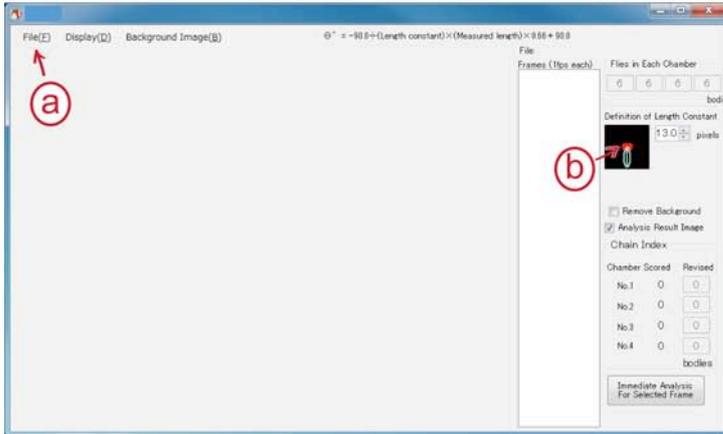
2. Start ChaIN

Double click ChaIN.exe

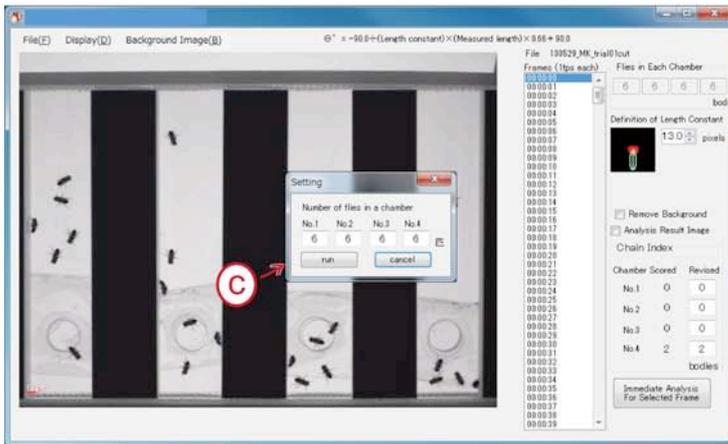
Open the AVI movie file you will analyze. Time resolution of the movie file should be 1 fps to get the correct timestamp.

After converting the movie file into binary image, ChaIN picks up 40 frames between 301st and last frames (during the “sound” period) randomly to generate a background image. ChaIN automatically detects four largest “white” areas as chambers, each of which would contain flies to be analyzed.

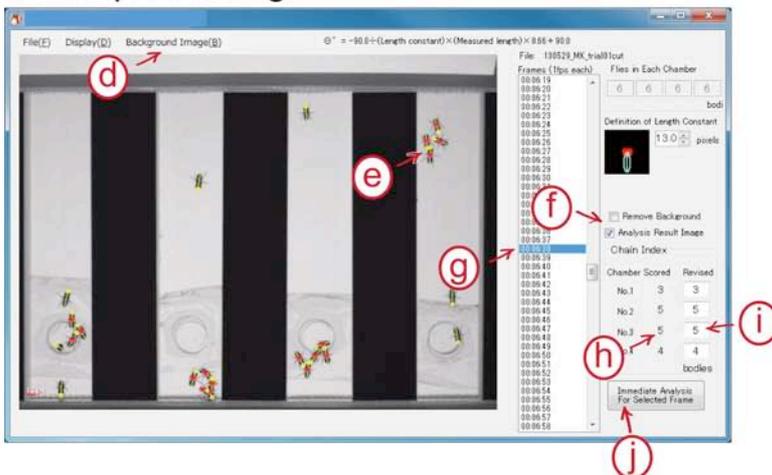
ChaN Initial screen



Start



Under processing



- Click "File" to open an AVI file
- Chain region defined by length and angle constants
- Enter the number of flies in each chamber

- d. Check, export, and import a background image
- e. Flies in chains are marked with red heart shapes
- f. Select an image type on screen
- g. Select a frame on screen. Note that “hh:mm:ss” shows a proper time only when the time resolution of the movie file is 1 fps.
- h. Chain index scored by ChaIN
- i. You can revise the chain index manually
- j. Analyze the frame on screen immediately

3. Export the result file

1. Chain index

Export the file as .csv file. File > Export > CSV File

2. Images

Export serial images to your folder. File > Export > All Image

Export an image on screen only. File > Export > Current Image

4. Background correction

1. When is it required?

ChaIN picks up 40 frames between 301st and last frames randomly to generate a background image. In case if flies do not move during this period, the silhouette of flies is regarded as a background. In such a case we recommend to correct the background image manually.

2. How does it work?

Save a background image. Erase the silhouette of flies manually using a graphics-editing program (e.g. Adobe Photoshop). Import the corrected image to ChaIN as a background image. Analysis will be automatically restarted.

5. Modify the constants

ChaIN.ini defines the constants L and k . You can change these values on this file.

1. How to change the Length constant (L)?

At the 1st sentence you can see:

ChainDefaultLength = 13.0; Length constant (len)

L is set to be “13.0” in this first setting. You can change the value as you want.

2. How to change the Angle constant (k)?

In the second paragraph you can see:

; Definition of the angle

; deg = -90.0 / len * L*0.66+ 90.0; L=|PCl

VerdictChain.Param1st = -90.0; First constant

VerdictChain.Param2nd = 0.66; Angle constant

VerdictChain.Param3rd = 90.0; Second constant

k is set to be “0.66” in this first setting. You can change the value as you want.

6. Tips

1. Setting a background image

ChaIN.ini defines other parameters such as the number of frames that are skipped for background calculation. In the initial setting ChaIN is set to skip the first 300 frames, which means 5 min if the movie file is at 1 fps. It is defined in the .ini file

SkipFramesForBackGroundCalculation = 300

You can change the number of frames to be skipped as you like.

2. Sample data description

We distribute a sample data at:

http://www.bio.nagoya-u.ac.jp/~NC_home/chain_E.html

In this movie file Canton-S flies were exposed to an artificial pulse song of 35-ms inter-pulse intervals between 5 min and 11.5 min. The time resolution of the movie file was down-sampled to 1 fps before starting ChaIN.