Sternberg Lab

Nematode Movement Analysis System

Instructions

Quick Instructions (Memory Jogger):

First, Capture Worm Data:

1. Tracker

2. Recognizer2.1 (with 'input' and 'params' input files on desktop)

then, Analyze using Matlab:

3. wormproc;

4. metrics6(0.0041, 240, 1, 'folderA', 'folderB', 'folder…');

5. histograms4('folderA', 'folderB', 'folder…');

# Capturing Worm Data

Tracker:

Tracker is used to keep a crawling worm within the field of view of the microscope camera for recording.

Power up the whole motion tracking rig:
- 'AVA' (the PC set up to assist with recording functions)
- VCR (rewind tape to beginning and reset counter)
- VCR Monitor
- Microscope light source
- Camera controller
- Bio-Point controller

Double click the Tracker icon on the desktop of AVA to start the Tracker program. An 'Automated Worm Tracker' window will appear that looks like the one shown at right.

Place a Petri dish with one worm onto the motorized microscope stage. Using the joystick, move the microscope stage to bring the worm into view in the Tracker window. Click the 'Enable Tracking' button while the worm is within the black outline in the Tracker window. As the worm crawls out of the outline the computer should move the microscope stage to bring the worm back to the center of the field of view. (Be sure to focus the microscope so the image on the VCR monitor and the Tracker window are sharp.)
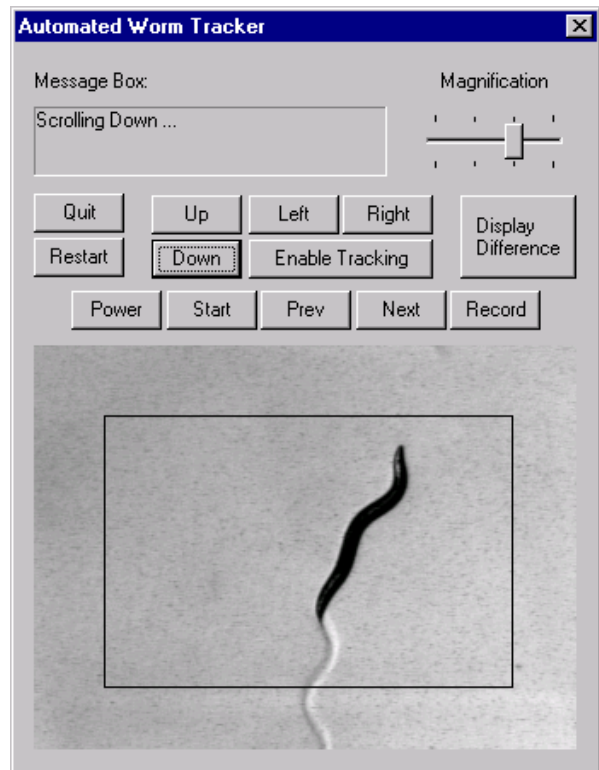
When ready to record, use the 'REC' and 'STOP' buttons on the VCR to begin and stop recording. Be certain to note the start and stop times shown on the VCR counter—these are necessary for the next step! Also, be sure to note the test conditions for each worm.

When finished recording a worm, click the 'Disable Tracking' button in the Automated Worm Tracker window to stop the tracking action, and remove the Petri dish.

When finished recording for the day you can shut down the
- Microscope power supply
- Camera controller
- Bio-Point controller
(These aren't necessary for the next step.)

Recognizer

Recognizer is a program that automatically plays back the videotape of crawling worms and digitizes the images to deduce the worm's positions and movements over time.

Recognizer needs two input files to do its job:
- <u>params</u> (the parameters Recognizer uses for digitizing your video, including **the name of the folder where you want Recognizer to put the digitized information**.)
- <u>index</u> (a list of VCR counter readings indicating where to begin digitizing, and for how long.)

Both files must be on the desktop of the computer connected to the VCR.

1. Edit 'params':

Double click on the 'params' icon on the PC desktop to edit 'params'. The editor window will list the following information:

```
foams = 3
foaws = 51
foass = 4
foathreshold = 0.80
bndryms = 15
bndryws = 281
bndrythreshold = 0.80
bndrysize = 50
spnsize = 13
saveimages = 100
outdir = C:\\Jane-AVA\\n1134\\TempDataBin1
savepoints = 1
pointsname = points
```

Most of these should stay as-is, but 'outdir' should be modified to list the folder where you want the digitized information to be saved.

> Important:
> a) Always use double-backslashes (even though MS Windows uses singles), and
> b) Never use spaces in the 'outdir' path name (even though allowed by Windows).

('spnsize' defines how many data points Recognizer should record along the length of you worm's body. Typically you will keep this set to 13.)

When finished editing 'params', save and exit.

2. Edit 'index':

Double click on the 'index' icon on the PC desktop to edit 'index'. The editor window will display information in the form of:

hour : minute : second : frame   recording-length (in minutes)

For example

```
0:00:30:0 04
0:05:30:0 04
0:10:30:0 04
0:15:30:0 04
0:20:30:0 04
0:25:30:0 04
0:30:30:0 04
0:35:30:0 04
0:40:30:0 04
0:45:30:0 04
```

('frame' will typically be left at zero, as shown in the example above.)

Edit these times to reflect the VCR counter readings for where to begin digitizing your worm recording, and the length of the sections to digitize (in whole minutes). Typically, if you recorded five minutes of worm movement, you will ignore the first and last 30-seconds (that is, using only the middle four minutes) to allow for any inaccuracies in the VCR counter, tape stretch, etc.

When you have finished editing the contents of 'index' to reflect your tape's start times, save and exit.

3. Start Recognizer:

Be certain that 0:00:00 on the VCR counter corresponds with the beginning of the videotape. (If you inserted a tape that was not fully rewound, rewind to the beginning and reset the counter.)

Reboot the computer (to free RAM), then double-click on the Recognizer icon on the PC desktop and go off to lunch! Recognizer will fast forward the VCR to the start times you specified in 'index', play the tape for the recording length you specified, digitize the worm's movements, and save the data in the folder you specified in 'params'. As Recognizer does its digitizing, you will see the worm video playing in a Recognizer window on the PC, with a 'spine' of points distributed along the length of the worm.

When Recognizer is finished, close the Recognizer window. (You can also shut down the VCR and VCR monitor.)

Recognizer saves the digitized information in a series of folders called worm1, worm2, worm3, … with worm1 corresponding to the first start time in 'index', worm2 to the second,… If you need to move (or rename) the worm__ folders into other sub-folders to better identify which is which, be certain to keep the worm__ folder names in the form:

wormX   (where X is an integer)

(If desired, you can add additional text to the folder name after the integer, provided you do not include any spaces in the name.)

Note: Each worm__ folder should contain a file called 'points' containing a list of xy coordinates for the worm's positions and a series of worm image files called file.1, file.101, file.201, file.301,… corresponding to the 1'st, 101'st, 201's, 301'st,… worm positions. These data are input to the worm motion analysis programs.

Troubleshooting Recognizer:

If Recognizer does not seem to operate properly or gives error messages, try these first:
1. Shut down and restart the PC.
2. Verify that there is sufficient space on the hard drive. You will need about 5 MB per four-minute worm recording. (Move files to another PC or archive files to CD to make room)
3. Verify that Tracker is not open when trying to run Recognizer. (Both programs try to gain complete access to the same digitizer card, so an open program will block the other program from operating.)

Analyzing Worm Data

Wormproc:

Open Matlab (double click on the Matlab icon on the desktop). The prompt (>>) in the Command Window indicates that Matlab is ready to accept input from you.

Start "Wormproc:

Wormproc is a master function that automates worm data processing.

Your objectives here are twofold:
   a) Verify that the computer correctly identified your worm's head-end
   b) Verify that bogus data is being ignored.

At the >> prompt, type *wormproc <Enter>* to begin processing worm data.
Matlab will prompt:
        Enter Directory name>
Type (or copy & paste) the name of the 'worm__' directory (<u>WITH THE PATH)</u>
containing files called 'points' and 'file.1', 'file.101', 'file.201', 'file301'…
   Example:
                C:\Jane-AVD\Arsenite-cat4-03-11-02\N2-0-NaAsO2\worm4 <Enter>

Wormproc will display a pair of blank editing windows: an editing window (to the right) and a reference window (to the left). (Note: windows will be blank until you start editing using the controls in the editing window.)

In the editing window:
   -   The top rows of buttons work similar to those on a VCR: Play>, Stop, Fast Forward ("fast" >>, and "faster" >>>), Rewind (again, "fast" << and faster" <<<).
   -   The two buttons labeled Step> and <Step move forward or backward respectively one frame at a time.
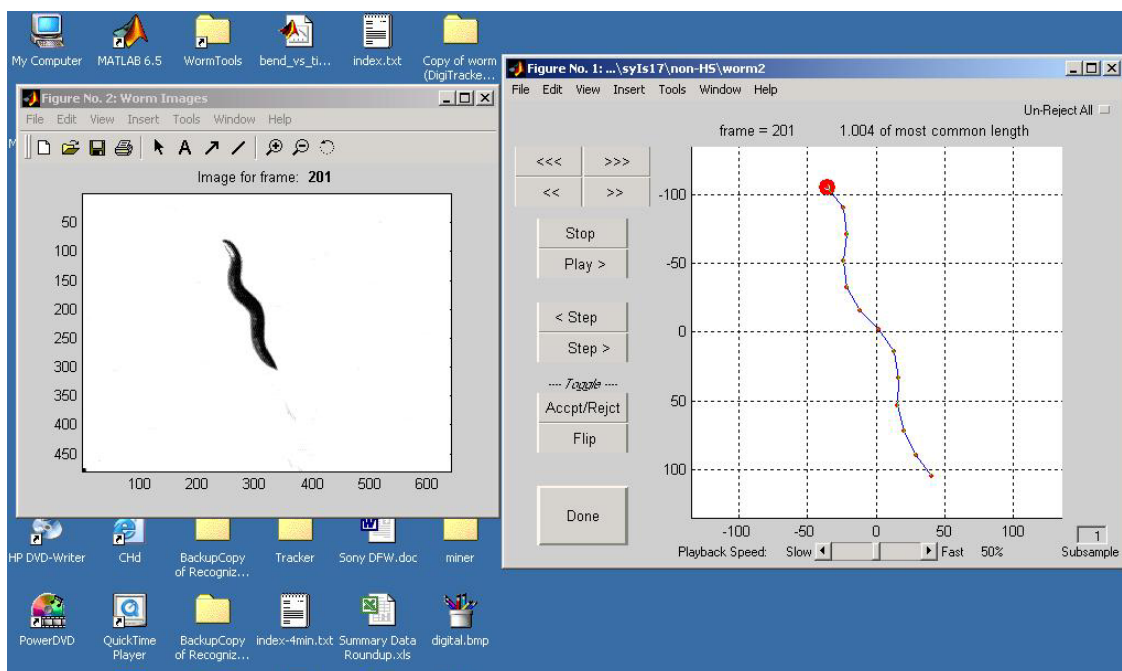
Using the mouse, click on the <Play> button in the editing window. A 'skeleton' worm (with a large red dot for its head) will move on the screen. Use the on-screen buttons to control the movie playback. (Don't forget about the "Playback Speed" slider at the bottom of the screen to speed up or slow down the playback to suit your taste – and your computer's processor speed!) At the same time, the reference window will display worm images captured from your videotape. These can be used as visual references to help confirm the orientation of a worm. (Note the Frame numbers listed at the top of both windows.)

Accept/Reject:
During playback of the movie Matlab will occasionally place a large red 'X' over the center of a worm skeleton on the editing window… This indicates a frame with missing data or a frame containing data that Matlab suspects to be invalid, for example if the worm's 'spine' becomes shorter or longer than Matlab thinks is likely. This is where the 'editor' comes in: there may be times when Matlab misses a series of frames that should have been rejected or conversely, rejects a series of frames that present obviously valid data…
- The button labeled <Accpt/Rejct> acts as a toggle to REVERSE the acceptance/rejection of the current frame AND ALL SUBSEQUENT FRAMES (all the way to the end of the 'movie'): all of the "Okay" frames (from the current frame onward) become "No Good" and vice versa. So, in the cases where you need to invalidate a series of frames that Matlab missed, simply forward to the first frame that needs to be rejected, clicks on the <Accpt/Rejct> button, forward to the first frame that now needs to be re-accepted, and click on the <Accpt/Rejct> button once more!
(For further discussion on how the toggle feature works, please refer to the Appendix.)

Flip:

Likewise, there will be times (particularly when a worm doubles back on itself) when Matlab becomes confused as to which is the worm's head end. In this case corrections are made with the button labeled <Flip>

- <Flip> works the same way as the <Accpt/Rejct> button but this time by reversing (head-to-tail and tail-to-head) the orientation of worms in all subsequent frames. (The toggle feature saves HUNDREDS of keystrokes on particularly uncooperative worms!)

When the 'movie' is completely edited, with all of the 'head-ends' depicted at the 'head-ends' and the valid frames accepted (and invalid data rejected), use the mouse to press the <Done> button. Matlab will automatically finish processing the data and will save the resulting data as a file called *data* in the worm__ directory specified at the beginning.

Repeat with any other worm__ folders, including those in other folders (for example for other drug conditions or other mutants).

N.B.: The "Un-Reject All" button in the upper right corner of the window removes all "reject" editing (except for missing worms) and resets the movie to the beginning. This button will typically only be used for exceptionally uncooperative worms.

Calculating Metrics

The Matlab function "*metrics*" uses the pre-processed worm data from "*wormproc*" to calculate measures of worm movement. The resulting metrics can be graphed or tabulated as needed.

From the Matlab command prompt type:
> *metrics6(mmpp, scnds, fpt, 'folder1, 'folder2,…)*

where
- mmpp is millimeters per pixel (0.0041 for worms recorded at 25x magnification)
- scnds is the length (in seconds) of the 'recognized' recording (For example, 240 for a 4 minute recording: 4 minutes * 60 seconds/minute = 240 seconds)
- fpt (frames per timeblock) is the number of frames (worm positions) to group together for the velocity calculation. That is, Metrics calculates velocity by looking at how far a worm moves every N'th frame, where N = fpt. Typically fpt should be 1 (calculating velocity for every frame).
- folder1, folder2,… are folders containing one or more folders named *worm__* (each containing a file named *data.mat*)

For example:
> *metrics6(0.0041, 240, 1, 'C:\Jane-AVD\Arsenite-cat4-03-11-02\N2-0-NaAsO2'); <Enter>*

or for multiple directories:
> *metrics6(0.0041, 240, 1, …<Enter>*
> *'C:\Jane-AVD\Arsenite-cat4-03-11-02\N2-0-NaAsO2', …<Enter>*
> *'C:\Jane-AVD\Arsenite-cat4-03-11-02\N2-1.25-NaAsO2', …<Enter>*
> *'C:\Jane-AVD\Arsenite-cat4-03-11-02\N2-2.5-NaAsO2', …<Enter>*
> *'C:\Jane-AVD\Arsenite-cat4-03-11-02\N2-5-NaAsO2'); <Enter>*

(Note: the three dots at the end of the lines above tell Matlab that you will enter more information. Also, the semicolon at the end of the last line tells Matlab not to print out intermediate gibberish to the screen.)

Matlab will prompt:
> `Display progress status?  (y)yes (n)no?`

Enter 'y' to see how long each sub-process takes, or 'n' for blissful ignorance.

Next Matlab will prompt:
> `Display coverage statistic charts?  (y)yes (n)no?`

Here, enter 'y' to have Matlab display charts showing how much of each worm's data is being used for calculating different metrics. (Or 'n' for more blissful ignorance.)

Matlab should then display something like:
> `Processing C:\Jane-AVD\Arsenite-cat4-03-11-02\N2-0-NaAsO2\worm1`

and should continue through with each worm in the specified folders. (This is a good time to enjoy a cup of coffee since each worm will take ~20 to 60 seconds.)

When *metrics* is done Matlab will again display the command prompt (>>).

Graphing Metrics:

To see the standard histograms of the processed data for sets of worms, from the Matlab command prompt type:

     histograms4('drive:\path…\folder1', 'drive:\path…\folder2'); <Enter>

For example:

     histograms4(…

          'C:\Jane-AVD\Arsenite-cat4-03-11-02\N2-0-NaAsO2', …<Enter>

          'C:\Jane-AVD\Arsenite-cat4-03-11-02\N2-1.25-NaAsO2', …<Enter>

          'C:\Jane-AVD\Arsenite-cat4-03-11-02\N2-2.5-NaAsO2', …<Enter>

          'C:\Jane-AVD\Arsenite-cat4-03-11-02\N2-5-NaAsO2'); <Enter>

Remember,

- You can graph the data for up to ten sets of worms on the same set of charts by specifying up to ten folders. (Note: More than ten folders will cause the line colors to repeat.)
- For a set of worms to be treated as a single line on the histogram charts, the 'worm__' folders should be grouped into the same parent-folder. For example, worm__ folders for all wild-type worms exposed to 1.25mM of $NaAsO_2$ from several days' recordings could be grouped together into a folder like
       'C:\Jane-AVD\N2-1.25mM-NaAsO2'.
- Each folder listed separately when starting the function 'histograms' will create a separate line on the resulting charts.

Be sure to edit the titles and legends to identify the charts. Do this by clicking the 'edit plot' arrow first, then double clicking the text to be edited. You can also add additional labels, etc., to the histograms, print them and save them to disk or CD. To allow the chart to print in color (or grayscale), be sure to click

     'Color (don't convert)'

on the 'Lines and Text' tab of the 'Page Setup' window.

(File | Page Setup… | Lines and Text)

Appendix

Additional help on understanding how the Accept/Reject toggle (and Flip toggle, too) works:

Note: A convenient analogy as to how the Accept/Reject toggle button works is to imagine a hanging ribbon with a green front and a red back… Imagine that most of the ribbon is green-side up, but that the ribbon is twisted in places to reveal sections with the red-side visible. In this analogy we can think of the green sections as "accepted" data and the red sections as "rejected" data. Further, we can think of the top-end of the ribbon as the first frame of our "worm movie" and the bottom-end of the ribbon as the last frame in our "movie". To "reject" a section of data we would twist the ribbon at the start of the new "rejected" section and un-twist the ribbon at the end of the new "rejected" section.