Dark Field Track-a-Worm Program

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Abstract

Caenorhabditis elegans, better known as C.elegans as seen in Figure 1, is a commonly used model system to study the functions of various gene products located at specific tissue, cellular, and synaptic foci to produce complex locomotor and bending behavior.[1] In order to investigate the behavior of this single millimeter long nematode, a tracking program is generally used.

Figure 1: Adult C. elegans

A tracker should be able to produce quantitative behavioral analyses by recording and analyzing a freely moving worm under a high magnification.[1] With a stereomicroscope, a camera, and a motorized stage, the Track-a-Worm program does just this, previously only possible in a bright field setting.[2] Given this preexisting Matlab-based bright-field program, a dark-field feature needed to be developed so that a moving worm with selected neurons labeled by a red or green fluorescent protein may be tracked.

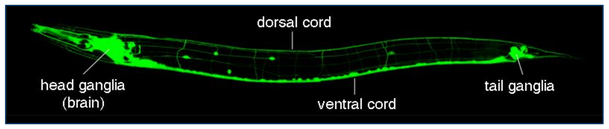


Figure 2: C. elegans nervous system with all neurons marked with green fluorescent protein (GFP)

Introduction

The code and design done for the Track-a-Worm software is entirely done within the program MATLAB. MATLAB is a high performance language for technical computing that integrates computation, visualization, and programming in an easy-to-use environment.[3] When dictated as the target folder, the Track-a-Worm Menu, Figure 3, will open upon startup. Here, the user has the option to choose between Calibrate, Record, Playback, Fit Spline, Analyze, Batch Spline, Batch Analyze, and Curve Analyzer. If starting the data acquisition from scratch, the first step is the calibrate module, then record, playback, fit spline, and analyze. The last three modules provide more extensive features explained in their descriptions below.

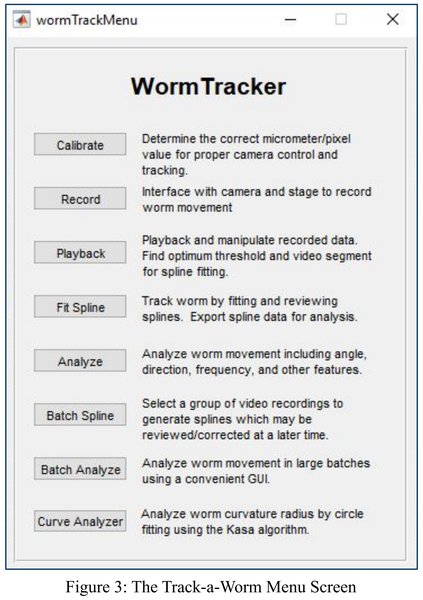
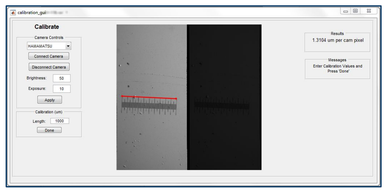


Figure 3: The Track-a-Worm Menu Screen

Features

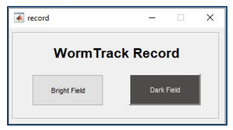
CALIBRATION

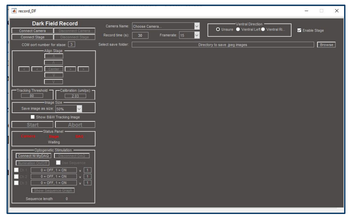
Calibration should be the first step to using Track-a-Worm. The purpose of the calibration module is to find the conversion factor from camera pixels to physical micrometers under the microscope. This will be helpful in the Record module.



RECORD

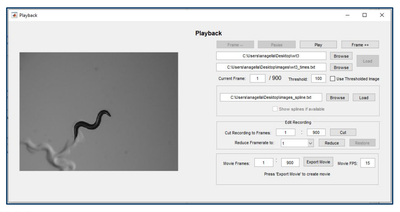
In this module, the user will be able to record in either bright field or dark field. The dark field tracker specifically uses the Hamamatsu camera to track specific neurons in C.elegans. Along with this camera, we used an image splitter that showed the frame with two filters, the green channel on the left and the red channel on the right. The module receives the position of the worm from the camera and tells the stage to re-center at 1-second intervals, finally saving the frame images to a selected folder. The user has the option to set recording aspects such as the frame rate and recording duration.





PLAYBACK

This module simply allows the user to view the recorded frames as a movie and determine a threshold needed to accurately see what is the background and what is the worm itself. It also allows the user to edit the video recording by cutting out frames.

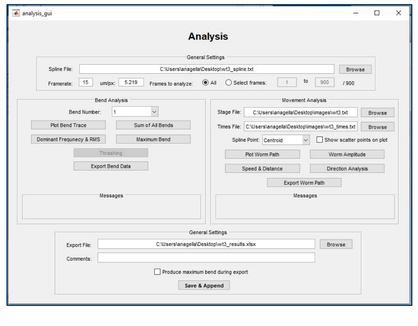


FIT SPLINE

The purpose of this module is to fit the splines of the worm images and identify the head versus the tail. The software can do it automatically, but it allows the user to go through the images and manually correct any mistake the program may have made. The Batch Spline module allows the user to choose multiple recordings to undergo automatic analyses which allows many recordings to be fitted overnight and then checked by the user the next day.

ANALYSIS

The Analyze module provides a plethora of information based on the images and splines acquired in the previous modules. For example, the user can see the worm path as the software takes the centroid of the worm from each frame and plots it against an x distance and y distance. Other valuable information given by this module are the speed, distance, bend, and amplitude of the worm. The user is then able to export and save this data to an excel file for an easier visual.



Major Changes

Bright Field vs Dark Field

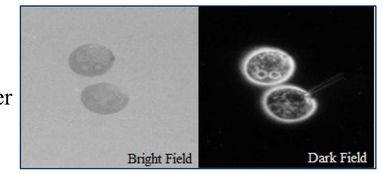


Figure 4: Bright Field vs. Dark Field Microscopy

A significant part of this project was to create a way to track and analyze the neurons of worms in a dark field. In order to do that, many changes needed to be made to the preexisting code. For example, in the recording module, the software for bright field tracking was able to find the location of the dark worm on a light background and use that information to move the stage to center the worm. In dark field tracking, the software had to do the complete opposite, by locating the bright regions of the dark background frame, as in the fluorescent proteins, and move the stage in a way that would ensure that these neurons would always be visible in the frame.

Converting from .bmp to .jpeg

An issue that arose with the preexisting software was the time it took to process and analyze the hundreds of image frames per each recording. A way to solve this was to change the file types of these images from .bmp, Bitmap, to .jpeg, Joint Photographic Experts Group. By saving as a .jpeg, the images are compressed, therefore faster to download and process. This also helped greatly by minimizing the amount of storage space needed to save the worm recordings. By using .jpeg, the size of the images became less than 2% of .bmp images .

Hardware

A major hardware change from the preexisting tracker is the usage of the *Hamamatsu Orca Flash 4.0* camera rather than the *Tisimaq* camera. A drop down menu was added to the Calibrate module and the Recording modules, giving the user a choice to use either the Tisimaq or Hamamatsu camera, depending on what was available to them. For every time the camera was called in the code, it would check what camera was chosen by the user and do that camera's specific set of commands. Also, for the dark field tracker, an image splitting device is needed to differentiate the low frequency red channels from the high frequency green channels for better analysis. The image splitting device used for testing is the W-View Gemini, and when connected to the Hamamatsu Orca Flash 4.0 camera, will preview frames split in half as seen in Figure 5.

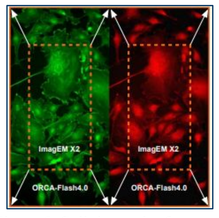


Figure 5: Split image by frequency

Discussion

Track-a-Worm is an extremely useful software when studying C. Elegans. The software provides a more accurate analysis of the worms than can be done with the naked eye. Not only that, it makes the process much faster, a valuable trait for when dealing with large quantities of worms. Although the software has many helpful attributes through its many modules, there are more features that can be added to make the program even better. In the future, further development of an intensity tracking feature will be helpful for the Dark Field Tracking. This feature should be able to track the brightest neurons in each of the green and red channels and plot their intensities over time.

References

1. Wang, Sijie Jason, and Zhao-Wen Wang. "Track-a-Worm, an Open-Source System for Quantitative Assessment of C. Elegans Locomotory and Bending Behavior." *PloS One*, Public Library of Science, 26 July 2013, www.ncbi.nlm.nih.gov/pmc/articles/PMC3724933/.
2. Wang, Zhao-Wen. *C Elegans Neurobiology Lab*, health.uconn.edu/worm-lab/track-a-worm/.
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4. Hutter, H. *C. Elegans Nervous System*, Simon Fraser University, www.sfu.ca/biology/faculty/hutter/hutterlab/research/Ce_nervous_system.html.