Lucas Junginger
UMID: 0032-4867

Tracking locomotion of larval *Drosophila melanogaster*

Introduction

I am majoring in Computer Science primarily as a complement to my major in Neuroscience. Over the course of my studies, I have seen many examples of computer-assisted data acquisition being an essential part to publications on a variety of research topics. I am interested in applying the knowledge learned in this class to further develop the data acquisition of my own research project in the Molecular, Cellular, and Developmental Biology department (Collins Lab). My project uses the fruit-fly *Drosophila melanogaster* to study various aspects of neuronal signaling and, in particular, axon degeneration and regeneration. One of the assays used at the lab struck me as underdeveloped and I intended on designing an algorithm to improve it. I am talking about a test in which many fruit fly larvae are placed on a single plate with a large circle drawn around all them and then monitored over a certain time. Once the time is up the plate is scored by the number of larvae that moved outside of the circle, giving an index of larval movement for a population of larvae; the index can be compared to that of different genotypes. My initial goal was to implement a program that reads in an overhead video of a larval population on a plate, identifies them as discrete larva, and tracks their change in distance over time, finally spitting out statistical information on the sample of multiple larvae. As can be seen from my report, I was not able to implement multiple object tracking for reasons I will discuss later; however I was successful in designing a program to track the movement of a single larva. The relevance of the finished product is dubious because the program was intended to analyze the movement of several larvae at once simply for the sake of convenience and precision; even though my program can correctly analyze the locomotion of a single larva, setting up an experiment with this method would require individual larvae to be systematically filmed and would defeat the purpose of simplicity. For this reason, I did not conduct controlled experiments to test the efficiency of my algorithm.

Approach

The first step to designing my algorithm was to optimize imaging parameters so to make object detection easier. I had to play around with different background colors to best enhance the contrast between the larva and the plate. Additionally, I needed a way to restrain the larvae so they would not escape. Some of the common materials used to hold larvae are shown below, transparent plastic dishes, as well as purple-tinted grape food plates. Incidentally, the plastic dish and the grape plate were both reflective surfaces that produced irregularities in the background. I experimented with colored tapes and found that these surfaces were considerably less reflective and produced a very consistent background. I attempted to coat one of the plastic dishes with tape however the background still appeared very uneven from the various overlapping pieces of tape. This wasn't the only problem: the larvae dry out on the surface of the tape because there is no moisture, and consequently stop moving. The solution

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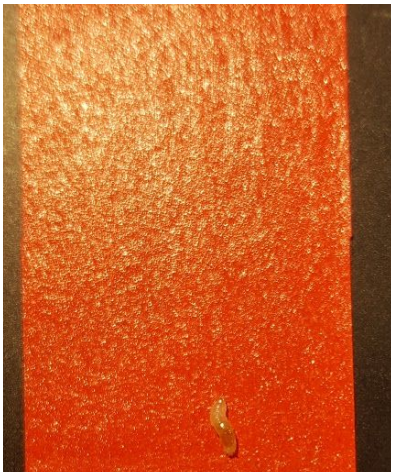
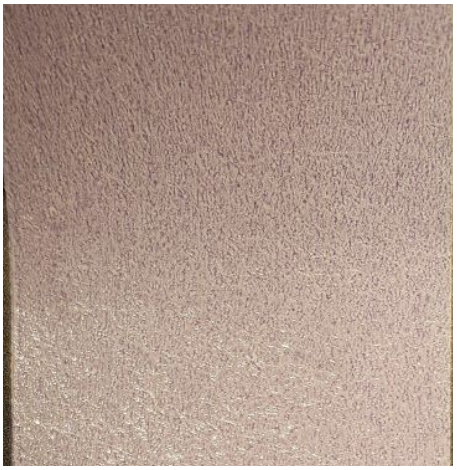
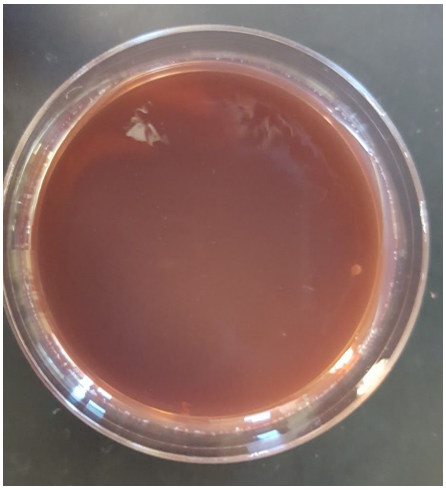
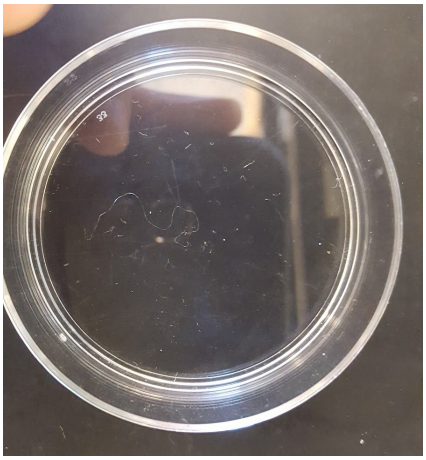
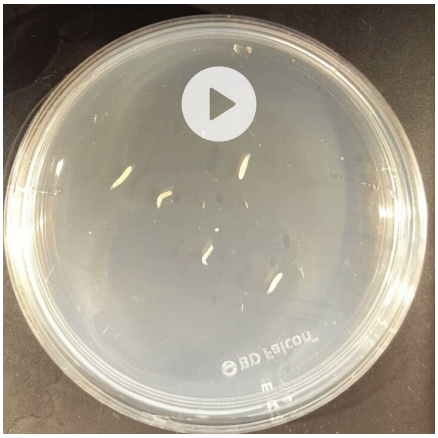
was to use a special transparent grape plate (shown below) placed over colored tape so to create a the contrast in background while still allowing the larvae to move.




In order: transparent food plate, green-blue colored tape, food plate over tape

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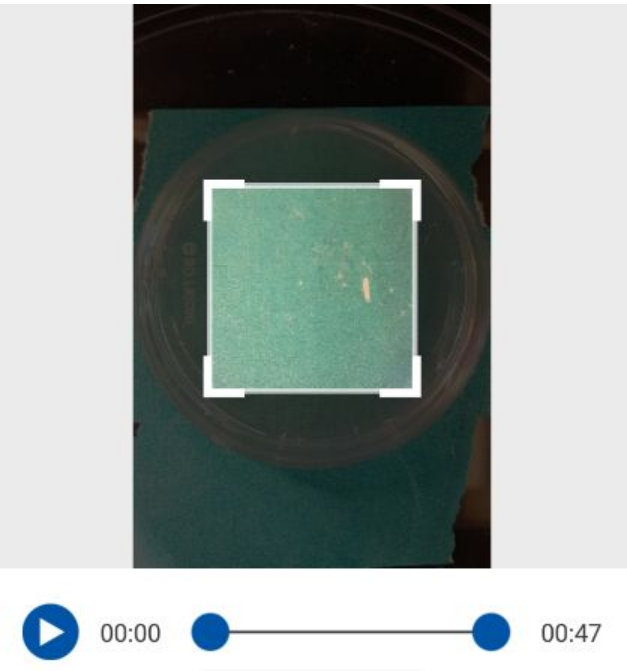
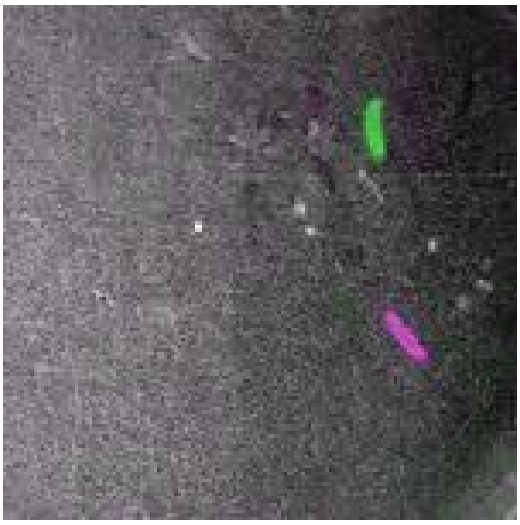
An important part of this experiment is for the data sets to be obtained consistently so that data can be compared. This meant that I had to keep the camera at a fixed distance from the larval plate; this was done by using the same stand to support the camera. Additionally, the pre-processing has the same between videos. The ultimate goal was to reduce the video's resolution to 150x150 pixels while avoiding any distortions between different videos. I used a free Android cropping software called *Video Cropper* to crop my videos into squares. To maintain the correct ratios between samples, I used the extremities of the larval to delimit the cropping region (left). This resulted in a roughly 613x613 pixels video which was then resized to 150x150 pixels (right; merged first and last frames of single.mp4). The MP4 file can be read into MATLAB as an array of images easily, which were then processed for object detection. Since my ultimate goal was to implement simultaneous video tracking for multiple larvae, I figured it would make sense to first implement the tracking algorithm for a single larvae.





Implementation

The core of my algorithm for single larva tracking involves iterating through the array of video frames and applying the Laplacean of Gaussian blob detector. In order to reduce the work, I set a hyperparameter which allows me to determine how many frames should be processed per real time second of video independent of . Because larvae move so slowly, I was able to lose resolution while maintaining the relevant information about locomotion, which performed a lot faster considering the slowness of kernel convolution. I did not have to resize my kernel because larvae are of an expected size; therefore I had to mess around with the blob detector parameters to convincingly detect larvae. Actually, the parameters were very rigid and

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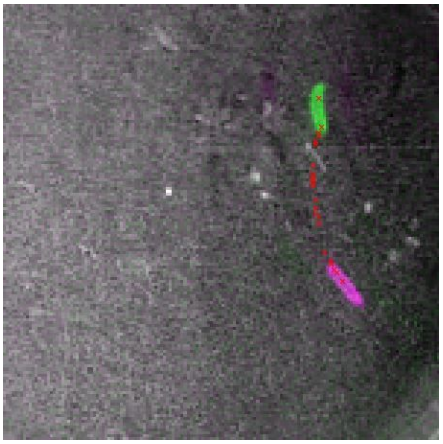
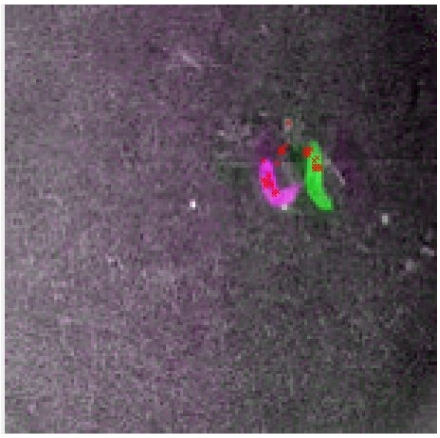
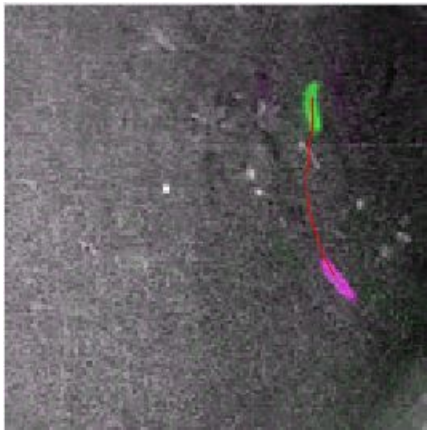
even at the best parameters I was getting multiple hits for a single larva. The object detector was not detecting objects centered on the larvae, likely because it was capturing both sides of the larvae as two different circles instead of detecting the larva as a single object. After applying 2D non-maximum suppression on each video frame with a kernel size, the highest scoring object was not actually centered on the larvae. To correct for this, I set a threshold value and averaged the position of the matches above the threshold after applying 2D NMS. The resulting coordinates were surprisingly accurate representations of the larva's position (below). I then plotted the larval trajectory and connected the larva's position at each frame sequentially to show its trajectory.

single.mp4

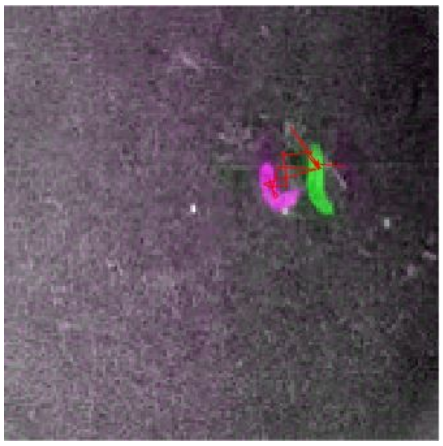


single2.mp4





single3.mp4



single4.mp4

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Experiments

The information of how many frames per second are being processed along with the coordinates of the larva at each frame allows me to calculate the total distance travelled, as well as average velocity of the larva. Below is information on the sample movies I collected from the lab. As was mentioned previously, this algorithm could still be used to attempt a fair experiment to test for movement deficiencies in larvae, though process of collecting many videos would ideally be avoided. Say for example I were to film 20 larvae from one genotype, then film 20 larvae from a different genotype with a movement deficiency: I could then generate data on which I would run T-tests to tell if there is a significant difference between the movement of one genotype to the other. There are some caveats to this setup, which I dealt with myself: there is a large variability in the amount of locomotion between larvae of the same genotype; also filming for too long usually means the larva leaves the field of view. These are both problems that would be better addressed by being able to track multiple larvae at once because the variability is drowned out in a large sample size and also the more larvae in a video means it would take less time of filming to argue that one population is statistically different.

Video (Duration in sec)	Total Distance Travelled (pixels)	Average Speed (pixels / sec)	Displacement from start (pixels)
single4.mp4 (44.25)	96.91	2.1902	31.2570
single3.mp4 (38.94)	89.20	2.2909	19.6977
single2.mp4 (63.58)	230.28	3.6220	26.3059
single.mp4 (30.73)	79.56	2.5882	62.5140

Conclusion

Lastly, my attempt at tracking multiple larvae failed because I was ultimately unable to extract a single convincing feature that represented a larva. The larva was recognized as multiple objects essentially surrounding the outline of the larva with similar LoG scores. After applying non-maximum suppression, the trajectory of the larva distorted because the highest object was not centered on the larva, but instead about N pixels away where N is the size of my LoG kernel. To address this problem I would need to develop a different kernel that is not so sensitive to circles but perhaps one that better represents the shape of the larva. I attempted to solve this problem by attempting to associate clusters of features inside a certain range with a certain larva, however once larvae move closer together their features start overlapping and the data is not specific enough to distinguish between features. Overall I was happy to be able to integrate both disciplines in this project and was frankly surprised with the progress I made.

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Given more time to research and learn about object detection, I believe this project could be truly finished.