An automated behavioral analysis of *Drosophila melanogaster*

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MAGGOT.py

YOUNG SCIENTIST PROGRAM
BLUE MARBLE SPACE INSTITUTE OF SCIENCE

BACKGROUND

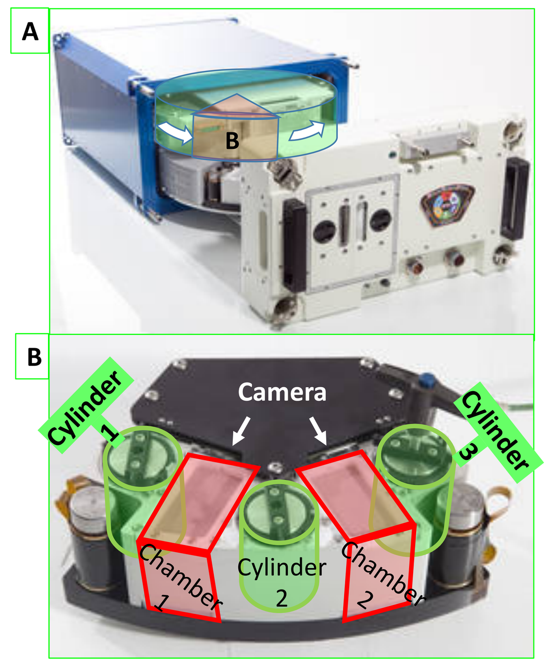
The behavioral characteristics of some animal models, such as *Drosophila melanogaster*, are strongly influenced by intrinsic and extrinsic factors and allow scientists to assess how changes in physiology or environment manifest into behavior. Conversely, assessing changes in behavior provides valuable information about how the physiology of that organism responds to external changes and serves as a readout of nervous system function. As part of this study, we analyzed the behavior of *D. melanogaster* exposed to spaceflight conditions. Larval behavior, specifically, was quantified after return from space by measuring crawling distance and number of full body wall contractions of each larva. Traditional manual quantification of these videos was strenuous and yielded varying results. From these studies we highlight a custom computer program, MAGGOT.py, designed to optimize data acquisition and processing. Using improved video recordings of wild type larvae and freely available libraries for Python we set parameters to compute distance traveled and number of peristaltic full body wall contractions (BWC) done by these larvae. Results show that our program is an efficient tool for analysis of larvae and adult locomotive behavior, thus providing scientists with a low-cost, efficient, and reliable method of quantifying behavioral data.

M.V.P. MISSION

Multi-use Variable-gravity Platform Validation mission (MVP mission) (SpaceX-14)
April 2nd – May 7th, 2018
The goal of this mission was to install and test the Multi-use Variable-gravity Platform (MVP) hardware, developed by Techshot Inc., on the International Space Station.

Summary of the Mission:

- Hardware houses 12 modules(B) arranged into two six-membered circular configurations (A)
  - First set of six were spun to replicate earth gravity
  - Second set of six were kept in microgravity conditions.
  - Separates flies between generations (earth vs space born)
- Returned larvae were analyzed through videos recordings in lab.
- Full Body Wall Contractions (BWC) were visually assessed in 3 separate increments of 30 sec
- Distance was calculated using an isometric grid
- ~800 animals were analyzed, amounting to ~100 hours of labor



Larval Behavior Results:

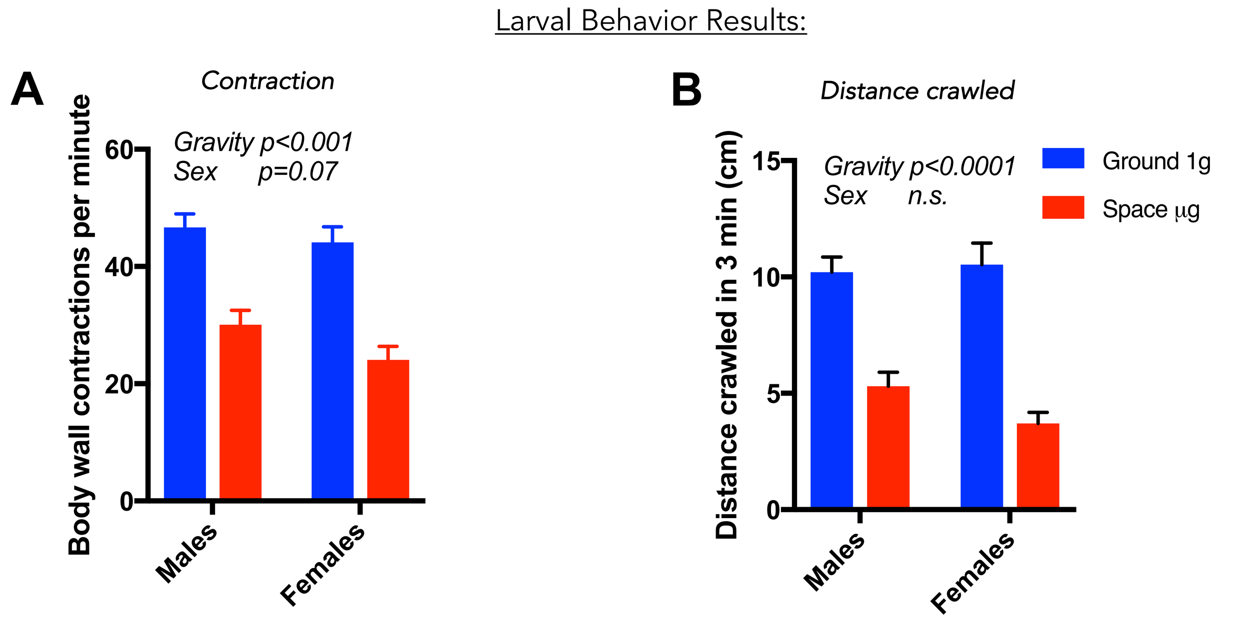


Figure 1. Neurobehavioral deficits in spaceflight larvae (A, B): Graphs showing decreased larval contraction and crawling distance in spaceflight larvae (both males and females) as compared to the ground controls using two-way anova.

CHALLENGES and OBJECTIVES

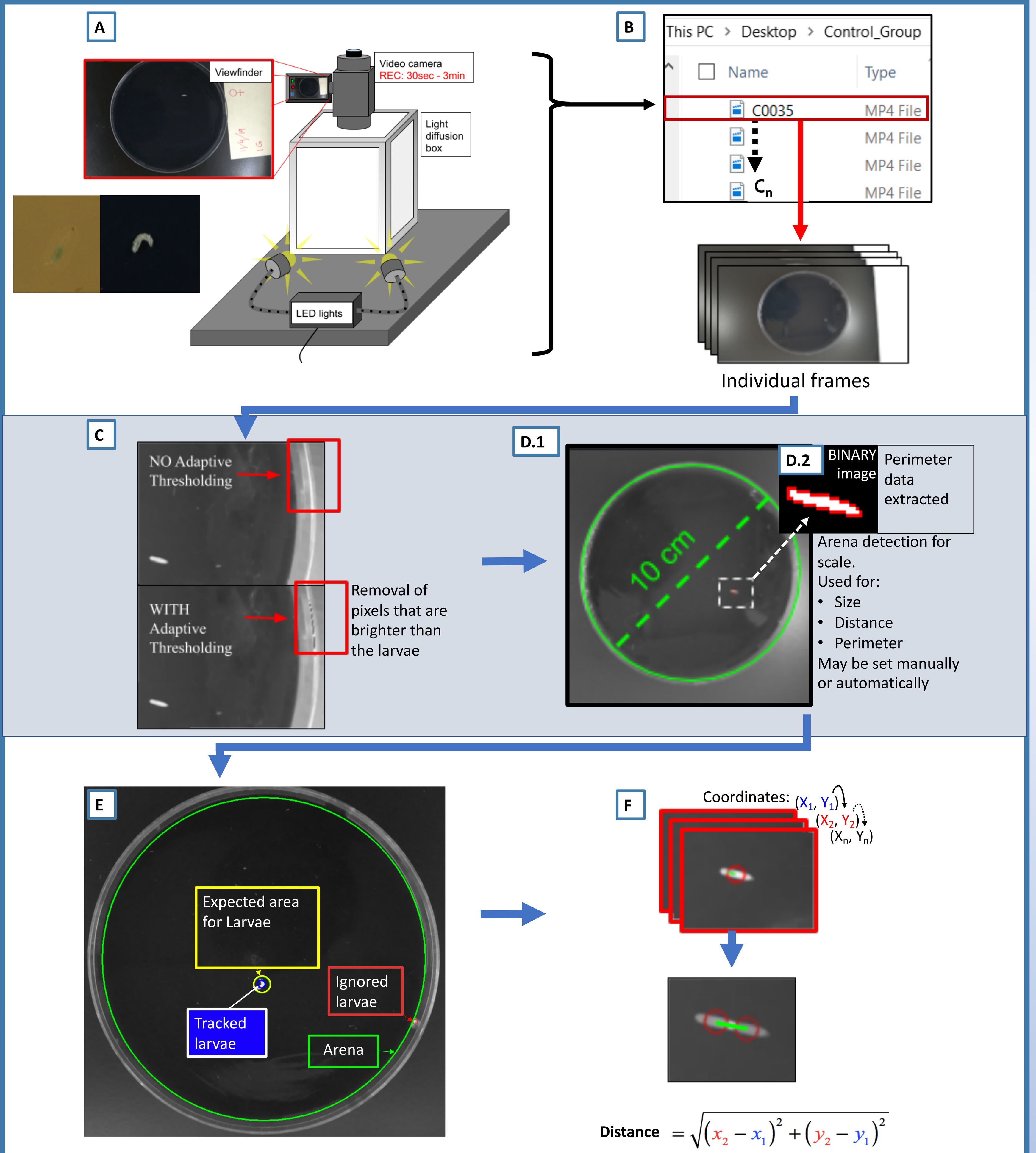
Challenges with traditional analysis:

- Inconsistent lighting
- Larvae was same color as agar
- Camera was not steady(Incubator)
- Variation between observers
- Observer fatigue
- Time-inefficient, thus costly
- Only segments of video are analyzed

Objectives

- Enhance protocol for recordings
- Write a program to automate the data collection
- Make that program user friendly
- Use only open source software
- Make that program open source

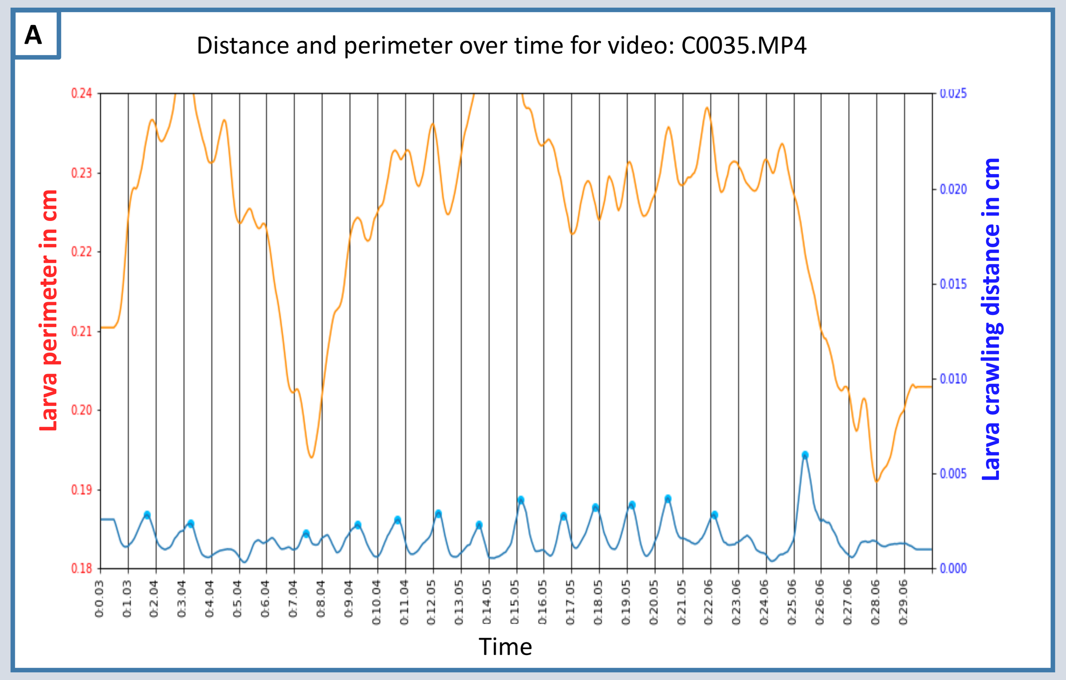
METHODS



$$\text{Distance} = \sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2}$$

Figure 2. *Flowchart of the automated program's essential functions: (A)Set up of larval recordings. 10cm petri dish is placed inside a paper box for evenly diffracted lighting. (B) Program iterates through videos and analyzes frames individually. (C) Frames are filtered to reduce white noise. (D.1) Circular shape of petri dish is detected for reference scale. (D.2) Frame is binarized and its perimeter is tracked over time. (E) Diagram of program running (F) Calculation of distance between frames.
*(GUI not included in flowchart)

OUTPUT!



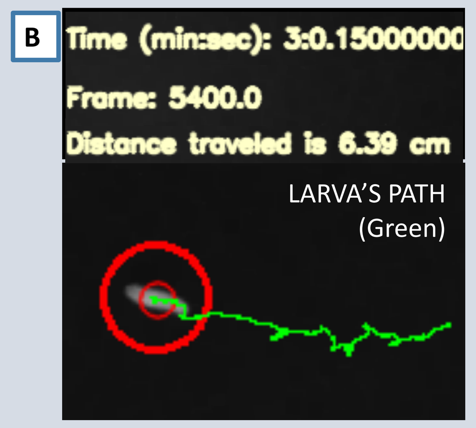


Figure 3. Example of Output data from the program: (A) Graph of Distance and perimeter over time. Blue peak markers indicate likely contraction. (B) Cropped image of larval path, distance, and time information saved after the program ends.

RESULTS

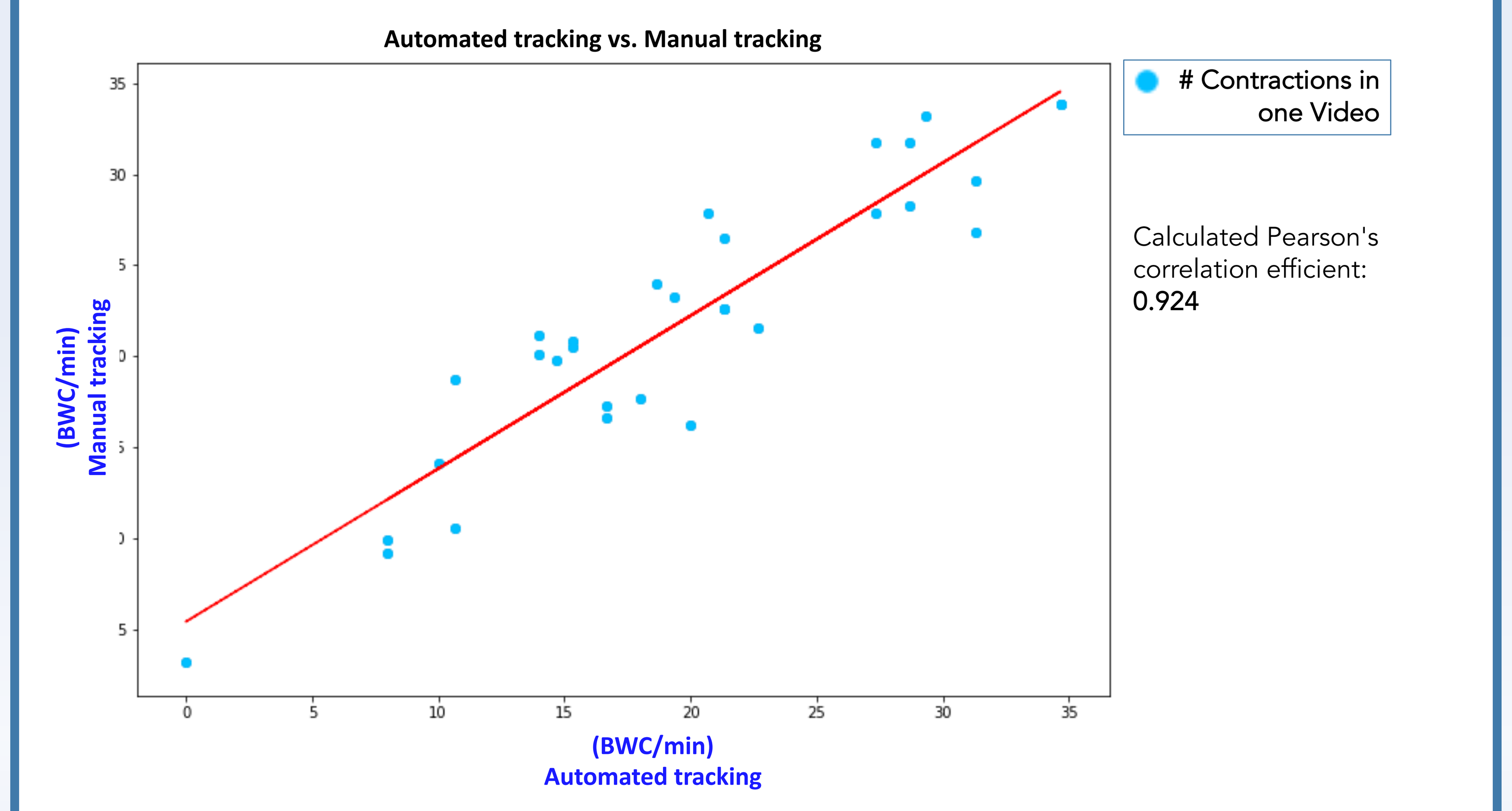


Figure 4. Automated tracking of Larvae correlate with Manual tracking: Graph comparing the estimated BWC from Manual and Automated tracking. Blue dots represent one video that was tracked for BWC.

SUMMARY

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Solutions to traditional analysis:

- Microscope light + light diffusion box (Fig. 2.A)
- Added activated charcoal to Agar for contrast
- Remains issue if recording in incubator
- Maggot.py reduces human error
- Maggot.py reduces need for manual tracking
- Maggot.py runs at real speed, freeing time
- Maggot.py analyzes any length of video desired

Objectives

- Enhance protocol for recordings ✓
- Write a program to automate the data collection ✓
- Make that program user friendly ✓
- Use only open source software ✓
- Make that program open source – *In Progress*

Limitations:

- Only tracks distance and body wall contractions.
- OpenCV is very difficult to install on certain devices
- Graphs require a lot of processing power, which varies across devices and should be rendered individually

Conclusions:

- MAGGOT.py serves as a useful tool for Neurobehavioral analysis of *D. melanogaster* Larvae yielding similar results to traditional Manual tracking
- MAGGOT.py offers a simple Graphic User Interface (GUI) allowing program to run on its own and saves user data.

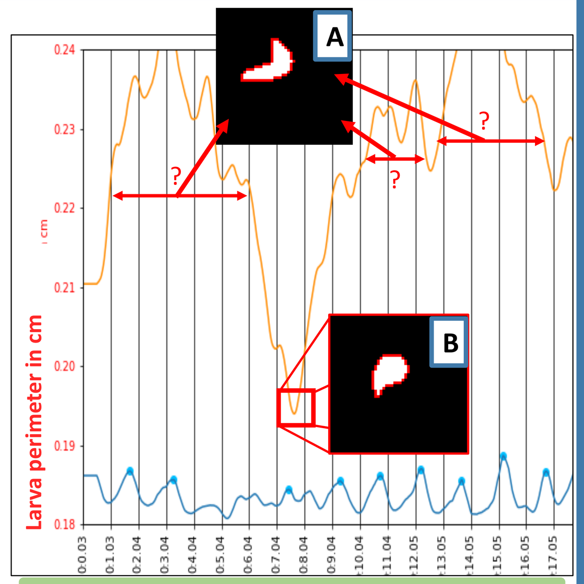

Figure 5. Motifs within change in perimeter over time that may correlate with head swinging(A) and curling(B).

What's next?!

- Certain patterns appear to correlate with curling and head swinging behaviors (see Figure 5 above). Further analysis, may identify parameters that envelop these motifs.
- Proof of concept
- Make open-source

ACKNOWLEDGEMENTS

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