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Original article

Automated Data Collection of *Drosophila* Movement Behaviour Assays Using computer Vision in Python

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Abstract

Drosophila melanogaster, commonly known as the fruit fly, is the ideal model organism to study behavioural genetics. It has been extensively used in studying many diseases. Many of those studies still use manual methods to assess the fly's behaviour under different conditions. In this article, we developed a method to track *Drosophila melanogaster* (both adults and larvae), and automate the process of data collection in larval crawling assay, and adult amputation assay.

Keywords: *Drosophila melanogaster*, Automating bahavioural assays, Computer vision.

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INTRODUCTION

The fruit fly, *Drosophila melanogaster,* has long been used in biological research projects since early 1900s (Resh & Cardé, 2009; Rubin, 1988). It is one of the best model organisms of choice to model diseases like neurodegenerative disorders (Lu & Vogel, 2009; Rosas-Arellano, Estrada-Mondragón, Piña, Mantellero, & Castro, 2018), cancer and cardiovascular diseases (Nichols, Becnel, & Pandey, 2012). The rationale behind using this model organism is its simplicity in its body architecture, considerably short generation time, easy husbandry as well as high similarity of its genes with human genes. Finally, its well-studied behaviour is among the best reasons behind using the organism for modeling diseases.

There are a number of methods that are implemented to assess fruit fly behaviour while studying such diseases. Such methods include RING (Rapid Iterative Negative Geotaxis) (Taylor & Tuxworth, 2019) and larval crawling assays (Nichols et al., 2012), Local and Global Thermal Nociception Assay (Chattopadhyay, A'tondra, & Galko, 2012), and leg amputation assay (Khuong et al., 2019) that is used to assess neuropathic sensitisation in *Drosophila*. These methods are continuously being modified to enhance the data collection and reduce the observation error. One of such enhancement is the multi-channeled RING apparatus used by (Gargano, Martin, Bhandari, & Grotewiel, 2005; Nichols et al., 2012) to contain a large number of flies with the same age and gender, thereby reducing variation while collecting data. Similar strategies are applied to larval crawling and leg amputation assays. However, since these are still manually calculated, the error rate of all of those approaches are limited to the human factor, to some extent. To get around this issue, it is best to automate the data collection using computer algorithms.

In order to further enhance the process of data collection from such assays, we aimed at automating the whole process by developing an algorithm that can be used for all the above mentioned methods. We achieved this by implementing computer vision technology using OpenCV (Bradski, 2000) library of Python 3 programming language. Hence, the objectives of this research project include:

1) Automating fly (larvae and adults) movement pattern tracking in Larval Crawling, RING and leg amputation assays.

2) Automate data collection and calculation of speed of individual flies from the tracked videos.

METHODOLOGY

Automating video tracking and data collection of *Drosophila melanogaster*

Currently, the process of data collection in behavioural genetics assays are mostly conducted manually (Eidhof et al., 2017; Simon et al., 2012), and thus it is both time consuming and error prone. An example of such manually processed data is shown in figure (**1**), in which the video recording is played several times and screenshots are taken at different instances, then the distances between different points are measured either using ruler or electronic measurements. For assays that include fly jumping patterns, the distance and speed of the flies cannot be accurately measured manually. Instead, the number of jumps a fly makes is counted then calculations are made as number of jumps/minute. Hence, it is best to use a computer vision algorithm to bypass such hurdles and accurately measure the difference between the control and the treatment sample sets.

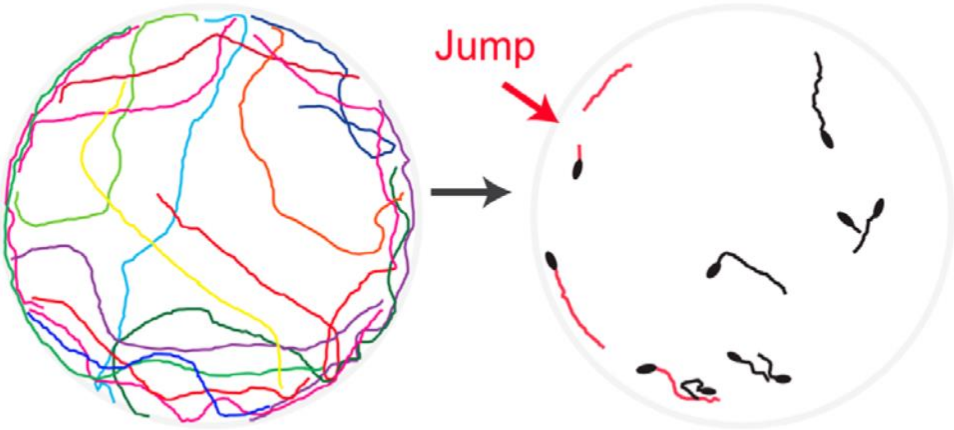


Figure 1. Leg amputation assay, manual annotation procedure. Taken from (Khuong et al., 2019).

By using python's computer vision library (openCV) we were able to develop a software that can track the distance traveled by individual flies being placed on a petri-dish for leg amputation assay. Injured and normal fly video tapes were taken from (Khuong et al., 2019), the method involves taking the initial position of the fly then calculating pairwise distances between each successive moves/frame using the following equation:

$x = (\text{sum}((x1\text{-}x2)\text{+ }(y1\text{-}y2))^2)^{0.5}$, where x is the variable that holds the output of the equation in every run of the test. x1,x2,y1,and y2 frame the position of the fly being tracked in the duration of the video recording, in real time. The Using this syntax in openCV, we were able to track and collect total distance migrated per individual flies in pixel per minute (**see the results section for more information**). After that, we recorded *Drosophila* larvae crawling on wet petri-dishes and assessed its walking speed using our software. Last but not the least, we tried to use the software to track flies in RING apparatus. For that purpose, a group of 6 to 10 adult, male flies were put in plastic falcon tubes and camera recorded then assessed for the validity of the software.

How the software works

The software is written as an open source module in python that contains one function. Once the function is called, it asks for the name of a video with its extension. Then, the video will be opened for the user to drag a square around the object that the user wants to track (in this case, it is the fruit fly, be it larva or adult). After testing it on many samples, it turns out that the software works best if the recording is carried out on a white background with minimum shading. Supplementary video (1) show the process of tracking a sample of *Drosophila melanogaster* after cutting its leg. The video recording was taken from (Khuong et al., 2019) and the procedure is carried out on the video using our algorithm.

RESULTS

Automated data collection of normal and injured flies

The following graphical representation shows how the flies are tracked by the algorithm as well as the output.

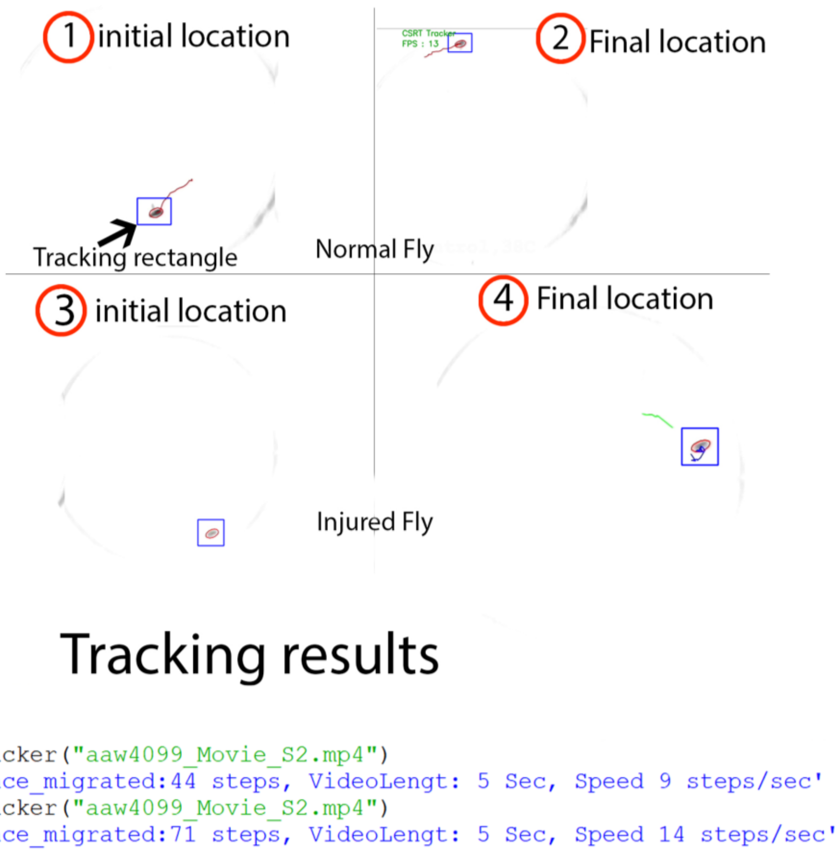
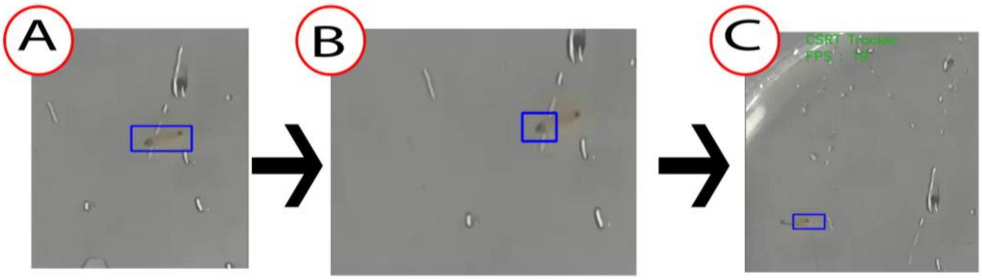


Figure 2. Automating data collection from Drosophila.

Figure 2 Automating data collection from Drosophila melanogaster leg amputation assay. As it is shown in the figure, the blue square drawn around each fly sets the algorithm to track the fly. Then, the analysis as shown in the tracking results section are provided as distance traveled and number of steps the fly makes to reach that distance with the speed of the individual fly. The normal fly traveled 44 mm in 13 sec and its speed is 3 mm/sec. However, the injured fly traveled 71 mm in a rather similar period of time and its speed is about twice as much as the control sample.

Automated Data Collection in Larval Crawling Assay

Following up the previous procedure, we tried implementing the algorithm on Larval Crawling Assay automation. Figure (3) explains the process of data input and tracking the larva sample.




```
>>> Tracker("Larva_Crawling_01_edited.mp4")
'Distance_migrated:11 steps, VideoLengt: 20 Sec, Speed 1 steps/sec'
```

Figure 3. Data input and tracking of Drosophila larvae, A) is the initial point of the larva, B) is when the larva starts to crawl, C) The end of the assay.

Since fly larvae move too slow, the speed parameter returned a small number, i.e. 1 step/second. We also tried implementing the algorithm on a grid background as it is shown in figure (4), but it lost tracking the fly due to extensive background noise. Hence, it is suggested to record the videos on a clean white background if attempted for such assays.

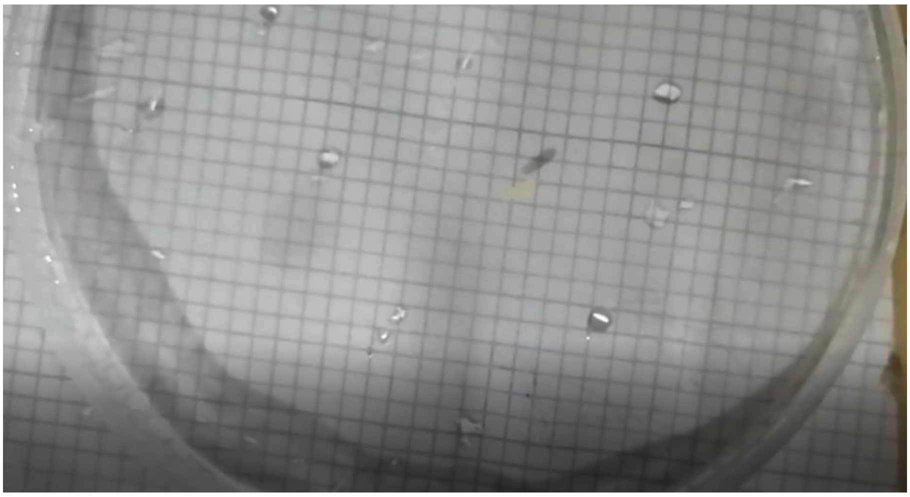


Figure 4. Larval Crawling Assay on grid background. The algorithm fails to track the larvae on grid background as the border lines stuck with the squares of the grid lines.

Comparison with manual method

In this study, we implemented automating data collection of certain fly assays using computer vision technology in python programming language. Manual methods have some drawbacks, such as being time consuming, error prone and often not reliable for large datasets. Hence, automating assays like leg amputation assay is of great importance.

Khuong et al., 2019 performed leg amputation assay manually, in which they took samples of flies (both injured and control samples) and counted the number of jumps per minute in each fly. They video recorded their sample sets and tracked the videos manually. Figure 1, shows the process of tracking fly movement in leg amputation assay, Figure 2 shows our automated method and the results we obtained after tracking the same samples they used in their assays. Our automated method has a difference with their manually annotated method. We track the fly movement in terms of total distance migrated in a specified duration of time and the algorithm does not depend on jumping behaviour alone, it rather considers the distance migrated including the jumping behaviour. On the other hand, the manually annotated method could be negatively impacted by several unknown confounding factors such as temperature, accuracy and precision of the observer, and health status of the flies, and jumping behaviour differences among the samples. Thus, one can rely on the automated method for better results and less time being consumed on performing the assay.

In addition to leg amputation assay, larval crawling assay is yet another one that is being conducted manually in many laboratories worldwide (Nichols et al., 2012). Figure 3 shows the process of automating larval crawling assay and how to collect data from *Drosophila* larvae without manually annotating their crawling. This way, one can use the same algorithm to automate data collection from larval crawling assay.

Discussion

Behavioural assays using *Drosophila* can be used to study many health related issues, including neurodegenerative diseases (Krench & Littleton, 2013; Prüßing, Voigt, & Schulz, 2013; Xiong & Yu, 2018), cancer (Read, 2011), and many infectious diseases (Dionne & Schneider, 2008). Pain perception is yet another area of research that has been recently studied using *Drosophila melanogaster* (Khuong et al., 2019). However, nearly all of the assays that are used to assess fruit fly's behaviour suffer from manual annotation errors and time consumption (Cao et al., 2017).

A better approach towards analysing data from such assay outputs is to use computer vision technology. In techniques like heat driven nociception assay, fruit flies that are injured tend to jump more often than a healthier one, this is due to their escape response behaviour (Khuong et al., 2019; Xu et al., 2006). The number of jumps/minute is one parameter used to assess the flies' noxious response to unpleasant heat, especially when injured (Khuong et al., 2019). Also, flies travel longer distances when they jump compared to normal walking. Hence, we accounted for the measurements of the total distance a fly makes when tracked by our software. As it is shown in figures (**1 and 2**), the injured fly travels just about twice the distance of a normal fly in the same duration of time. Thus, the software can be used to assess this behaviour efficiently and accurately.

From the data science point of view, some behavioural assays that aim at monitoring movement pattern during recovery often include injuring flies and then counting the number by which any given fly jumps per minute (Khuong et al., 2019) or observe the walking pattern changes during recovery period (Isakov et al., 2016). The former parameter does not tell the qualities of the jumps by the flies and it is difficult for feature extractions later on when attempted to model diseases mathematically. However, calculating the distance that the flies travel per a duration of time provides more information about the health status of the flies, and enables scientists extract more features for disease modeling purposes.

Another reason for implementing computer vision in this project is that openCV library is now integrated with TensorFlow, which is a fabulous unsupervised machine learning library. This computer vision technology along with machine learning would provide even better understanding to *Drosophila* behaviour when modeled for different diseases, which will in turn further enhance our understanding to human diseases. This can be achieved by unraveling hidden patterns through implementing even other unsupervised machine learning technology such as clustering algorithms (like principle component analysis) and feature extraction. Consequently, using fruit flies will be even more valuable to broaden the horizon of our knowledge about human diseases and disorders.

Conclusion

To sum up, *Drosophila melanogaster* is one of the widely used model organisms for its simple body architecture and ease of use in the library. There are many assays being developed to study the fly's behaviour, most of them are manually annotated. In this study, we tried to automate the process of data collection and feature extraction by calculating three parameters, which are number of steps a fly makes, duration of the recording and speed of the fly in that time. The source code of this work will be made available as an open-source project for further development and tailoring by other scientists in the field of behavioural genetics.

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