Automated Categorization of Drosophila Learning and Memory Behaviors using Video Analysis

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Introduction

- The goal of this project is to create an automated method for characterizing the learning and memory behavior of fruit flies by analyzing video of their movements.
- The ability to study learning and memory behavior in living organisms has significantly increased our understanding of what genes affect this behavior, allowing for the rational design of therapeutics in diseases that affect cognition.
- The fruit fly, *Drosophila melanogaster*, is a well established model organism used to study the mechanisms of both learning and memory *in vivo*.
- The techniques used to assess the behavior of flies, while powerful, suffer from a lack of speed and quantification.
- The method is being developed to replace and improve a labor-intensive, subjective evaluation process with one that is automated, consistent and reproducible; thus allowing for robust, high-throughput analysis of large quantities of video data.

Pipeline

- An input video contains 3 to 6 cells; where each cell holds a pair of male and female flies.
- Individual frames are extracted from the video.
- The frames are cropped to isolate each cell.
- Individual flies are identified in all of the frames for each cell.
- The area of each fly is calculated.
- The flies' motion are tracked.
- Once the flies are identified and tracked, various geometric measures are computed.
- The measures are computed for numerous experimental samples and produces a high dimensional feature vector that quantifies the behavior of the flies.
- Clustering techniques, e.g., k-means clustering, may then be applied to the feature vectors in order to computationally classify each specimen.

The pipeline of the process:

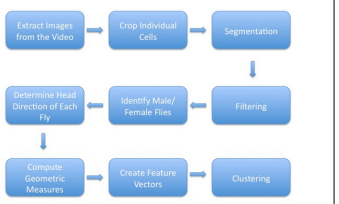

Figure 1: Pipeline of the Process

Segmentation and Filtering

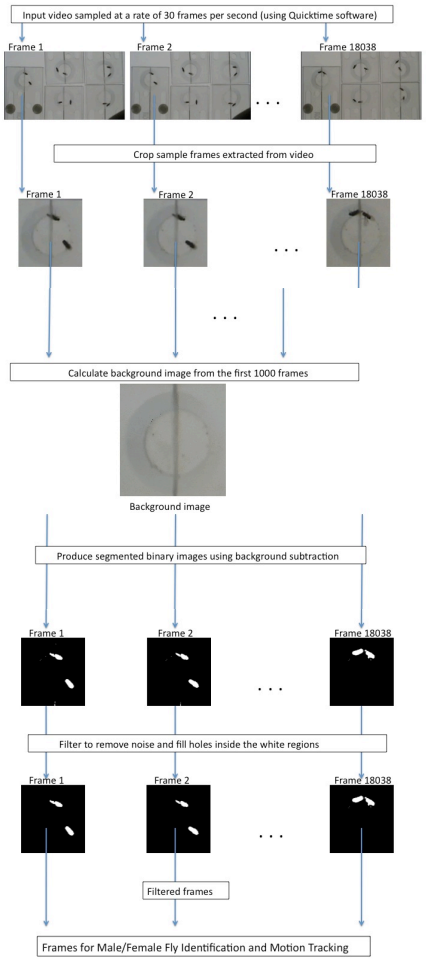

Figure 2: Segmentation process with filtering

Identifying Male/Female Flies

- A sequence of approximately 18,000 frames, extracted from a video clip, is divided into subsequences after filtering out the noise and filling the holes inside the white regions.
- Male and female flies are identified for each subsequence (greater than 15 frames) where the flies are separated.
- A starting frame is identified in a subsequence. In this frame the distance between the flies is maximum over all frames in the subsequence.

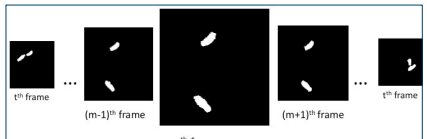

Figure 3: m^{th} frame is the frame where the two flies are furthest apart in the subsequence between s^{th} frame and t^{th} frame

- The two regions are tracked by using a frame-to-frame minimum distance assumption.
- The average area of each region is computed over the frames of the subsequence. The region with the larger average size is assumed to be the female fly.

Identifying the Head Direction

- The velocity vector (VV) and the central axis (CA) in the direction of VV is computed for all the frames in a subsequence for both flies.
- The longest positive dot product series of the CAs in the subsequence is computed.
- The frame with the maximum speed in this series is chosen as the starting frame.
- The head direction vector (HDV) of the starting frame is the CA in the velocity vector direction.
- The HDVs in remaining frames are computed from the starting frame's HDV.

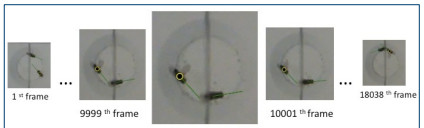

Figure 4: Sample output after fly identification with tracking

Geometric Measures

We compute 5 Geometric Measures:

- While the flies are separated, the percentage of frames when one fly is looking at the other fly. We assume that the male fly follows the female fly.
- Percentage of frames when the flies are together.
- Distribution of the Centroid Distances between the flies.
- Distribution of the Head Direction Angle between the flies.
- Distribution of the flies' Speeds.

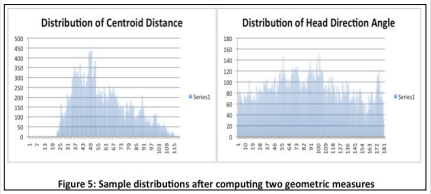

Figure 5: Sample distributions after computing two geometric measures

- The mean and standard deviation are computed for each distribution.

Clustering

- Three types of video clips of 10-minute duration are processed. These are: first 10 minute (First10Min), last 10 minute (Last10Min), and immediate recall 10 minutes (ImmediateRecall10Min). For some fly-pairs, we only have the immediate recall video clips.
- The feature vector consists of 8 numbers: % Frames Fly Looking At, % Frames Flies Together, Mean of Centroid Distance, Std. Dev. of Centroid Distance, Mean of Head Direction Angle, Std. Dev. of Head Direction Angle, Mean of Speed x 10, Std. Dev. of Speed x 10.
- K-means clustering is used for automatic grouping.
- 2-means clustering was run on the difference of First10Min and Last10Min feature vectors.
- The input set consists of 10 samples.
- The 10 samples were consistently grouped into 2 classes. The first class corresponds to wild type flies. The other corresponds to diseased flies.

Future Work

- Process and analyze additional video clips.
- Perform clustering on individual measures.
- Define a single quantity based on our geometric measures that is equivalent to the current Courtship Index (CI) used to characterize fruit fly behavior.