Inexpensive automated *Drosophila* geotaxis & phototaxis assay with real-time data analysis.

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Introduction

Traditionally the simplest and most common *Drosophila* assay involves geotaxis and phototaxis. This assay is used to determine *Drosophila's* ability to climb up a surface toward a light source. The conventional method uses a capped graduated cylinder, a flashlight, and a stop watch. In a darkened room the cylinder is tapped to ensure the *Drosophila* are dislodged to the bottom of the cylinder. The flashlight is then turned on, and a timer is started. The user determines the time points and number of flies able to climb past a specific threshold distance. Issues with this method are numerous. For example, conventional flashlights do not provide a consistent light source as battery power decreases overtime, and the amount of ambient light in a room is not consistent from assay to assay. As the cylinder is tapped down by hand at the beginning of each assay, there is a large degree of variation from user to user, which could result in variations in fly recovery time. Most notably as the assay relies on visual observation, measurement accuracy is highly variable from person to person.

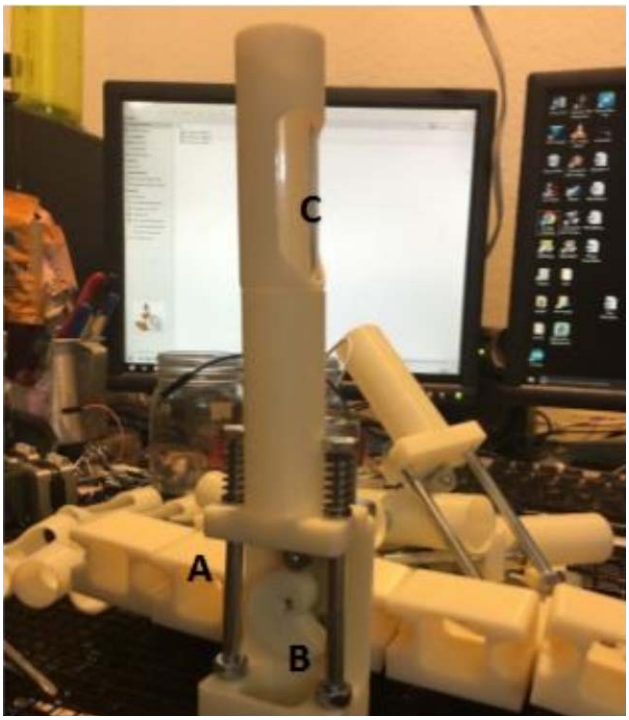

Figure 1. Motor cam and cylinder holder. (A) 4" × ¼" spring loaded bolts guide rails. (B) Snail cam with cabinet door roller. (C) Cylinder holder and viewing port.

Methods

An inexpensive automated method was developed after several design iterations to counter these issues found in the manual method geotaxis and phototaxis assay. The automated assay uses a full enclosure to ensure consistent ambient lighting levels. The plastic enclosure consists of 1/8th inch high strength ABS plastic, heat formed and later 3D printed to fully enclose the automated assay.

An RGB LED replaced the flashlight as the light source used to enable phototaxis (Figure 3 C). RGB LEDs can provide red, blue, or green light as well as true white or any shade between to allow for color specific phototaxis assays. Unlike the flashlight, the LED light is of constant intensity due to direct wiring to a consistent power source. The fly cylinder is tapped down via a motor cam mechanism to ensure consistent force for each trial. Earlier iterations of this system used a vibration mechanism to dislodge the flies, but this resulted in increased fly recovery time (Figure 1 A). The cam mechanism is made up of 4" × ¼" bolts with ¼" springs and nuts. The roller is a cabinet door roller (Figure 1 B). These components can be found at any hardware store. The cam mechanism frame and sample holder are made of 3D printed ABS

plastic. An inexpensive Logitech HD C270 webcam was stripped of its casing allowing for adjustment of its focal length and removal of its infrared filter (Figure 4 A). This allows the system to record high definition close range images in low light of the flies as they climb beyond a 10 cm threshold mark. The camera was then mounted to a 3D printed L bracket in front of a viewing port on the cylinder holder (Figure 1 C). An Arduino UNO was used as the controller for the RGB LED and cam motor (Figure 3 B). A solid-state switching circuit was created to allow for interfacing the UNO controller to the cam motor. The UNO and C270 were routed to a USB 3.0 hub (Figure 3 A). This allowed for additional bandwidth for peripherals such as a vacuum optical fly counter to be piggybacked through a single USB 3.0 port for PC control. The USB hub also allowed for wiring of power switches to act as manual resets to the C270 and UNO components. Additional power was supplied via a 9v DC power supply.

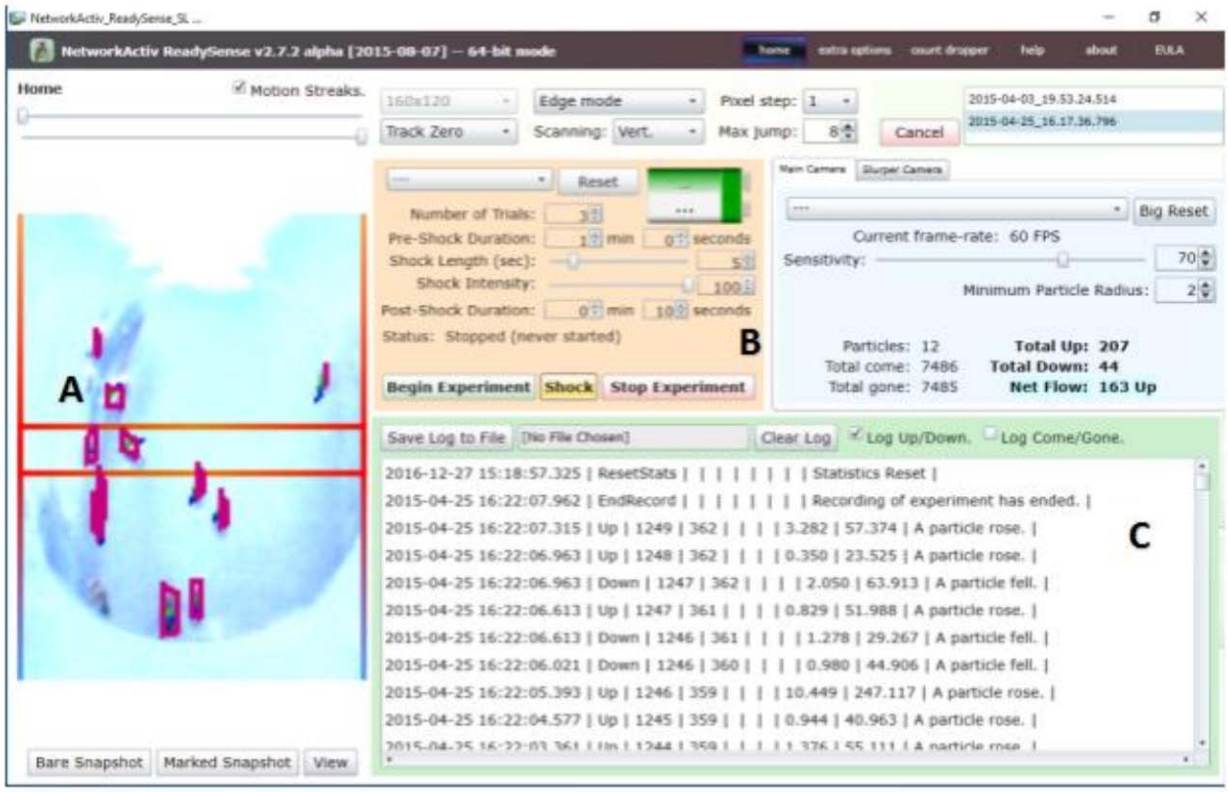

Figure 2. ReadySense user interface. (A) Video feed with object tracking and 10 cm threshold marker. (B) Assay settings menu. (C) Raw data log area.

Software called ReadySense was developed by NetworkActiv to control this system (Figure 2). The software interfaced with the Arduino UNO controller allowing for computerized control of RGB LED spotlight intensity and color balance. The motor cam mechanism is controlled as well allowing the experimenter to set the duration of tapping (Figure 2 B). The software allows the experimenter to set the number of assays to run, as well as the duration and timing of each assay. The software interface with the C270 webcam allows for control of camera settings as well as video capture. A user defined detection region and threshold level can be set to allow for bidirectional counting of flies passing the 10 cm mark in either direction (Figure 2 A).

The software analyzes video capture frames in real-time, or optionally post-experiment. Two modes of object detection are provided: Mass Mode (pixel cluster density) or Edge Mode (contiguous region), to accommodate different lighting, resolution, and processing power circumstances. Detected objects (flies) are assigned identifier values in order of appearance, and their telemetry (location, speed, and direction) is tracked, allowing flies temporarily to obstruct one another while crossing paths without interfering with motion

tracking. Detection thresholds can also be controlled by the experimenter to allow the software to track objects within a specific size range, and automatic debris management ignores unmoving anomalous spots or regions in the image. The software can process telemetry data both in real-time and from prerecorded video files allowing for rapid and high throughput analysis (Figure 2 C). All data are then outputted to an Excel file for easy analysis. Assay settings are saved automatically to allow quicker experiment replication with less chance of misconfiguration.

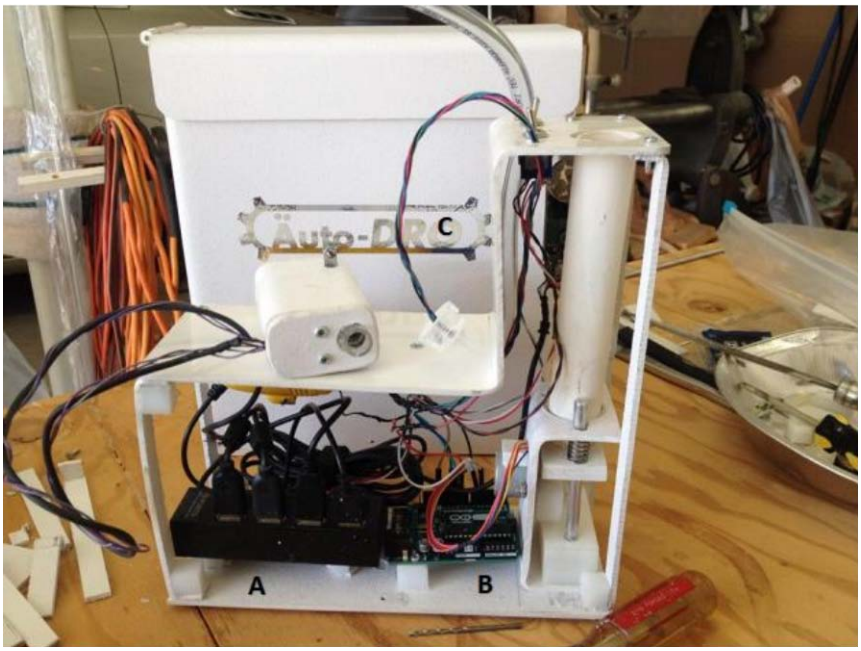

Figure 3. "AutoDRO" assay internal configuration. (A) USB 3.0 hub modified with manual switches. (B) Arduino UNO controller. (C) RGB LED spotlight.

Figure 4. C270 HD webcam on L bracket. (A) Logitech C270 HD webcam with modified focus and removed IR filter.

The software also provides additional modules to interface with systems for vacuum optical fly counting and sugar preference assay image analysis.

The automated *Drosophila* geotaxis & phototaxis assay nicknamed “AutoDro” in conjunction with the custom software ReadySense allows for consistent reproducibility regardless of the experimenter. The ABS outer casing allows for consistent control of ambient lighting. RGB LEDs allow for consistent control of light source intensity and color balance. The motorized cam mechanism allows

for mechanical tapping of the cylinder. The C270 HD webcam allows for precise recording of *Drosophila* movement. The software enables real-time or post-processing of video allowing not only for counting and recording of threshold crossing time points, but also for other data such as speed. Without the need for a human observer, trials may span extended lengths while maintaining repeatability. All assay data are automatically outputted, further reducing the possibility of human error.