*Journal of the American Mosquito Control Association,* 26(2):167–171, 2010


# QUANTIFYING THE MOVEMENT OF MULTIPLE INSECTS USING AN OPTICAL INSECT COUNTER

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ABSTRACT. An optical insect counter (OIC) was designed and tested. The new system integrated a line-scan camera and a vertical light sheet along with data collection and image-processing software to count flying insects crossing a vertical plane defined by the light sheet. The system also discriminates each insect by its position along the horizontal length defined by the light sheet. The system was successfully tested with a preliminary experimental protocol for determining whether groups of flying mosquitoes preferred or avoided attractants and repellents in a flight tunnel. The OIC counted the number of mosquitoes that crossed the light sheet and recorded the horizontal position and time each insect passed through the light sheet. The system provides a straightforward and reliable method for measuring and recording spatial and temporal information for insects that pass through an established plane.

**KEY WORDS** Optical mosquito counter, light sheet, insect detection, insect behavior

## INTRODUCTION

Automating the entomologically important process of insect counting has always been of keen interest. Early electronic insect counters (Lowe and Dromgoole 1958, Keighley and Lewis 1959, Hughes and Woolcock 1963) counted insects suspended in a fluid and were not suitable for use with live insects. Pearson et al. (1975) described an inexpensive electronic insect counter that was more suitable. The apparatus electronically counted up to 200 mosquitoes per minute as they were transferred from one container to another by a gentle airstream.

An Electronic Grain Probe Insect Counter (EGPIC) is a patented (no. 5646404, 1997) method that provided automated real-time monitoring of insects by using modified grain probe traps (Shuman et al. 1996, Litzkow et al. 1997). The EGPIC system provided real-time/monitoring by using an infrared beam to detect and provide a time-stamped count for each insect entering any one of an array of modified grain probe traps distributed throughout bulk-stored grain. The EGPIC's design has been modified several times (Shuman et al. 2001, Epsky and Shuman 2002) because of extensive testing that discovered performance problems under harsh field conditions (Brenner et al. 1998, Arbogast et al. 2000, Epsky and Shuman 2001, Toews et al. 2003). Shuman et al. (2004) described the design, development, and performance of a new patent-pending invention that enhanced the performance of the previously described EGPIC system by identifying the species of detected insects as well as rejecting erroneous counts by using quantitative analysis of the infrared sensors' analog output signals.

The objective of our study was to design and implement a system that measured and recorded many insects in flight and record spatial and temporal information as they passed through an established plane. The motivation behind the optical insect counter (OIC) was to develop a system that could be to track mosquitoes used in tests of attractants and repellents in flight tunnels.

## MATERIALS AND METHODS

A Plexiglas® insect flight tunnel (Raina et al. 1986) at the Henry A. Wallace Beltsville Agricutural Research Center, Beltsville, MD, was used in this study. The tunnel was 3.0 m long with a 60-cm-tall horseshoe-shaped cross section. The ends of the tunnel were enclosed with standard screen-door mesh screen. A round hole was cut in the center of screen at the downwind tunnel and fitted with a 7-cm-diameter plastic collar 16 cm from the tunnel floor to serve as a mosquito release point. The tunnel's interior was accessed by 3 piano-hinged doors located along the tunnel's side. The 3 m × 58.4 cm plexiglass floor of the tunnel was supported on a wood frame. The transparent width of the tunnel floor was 40 cm. One end of the tunnel was connected to a 12 $m^3$ chamber by a cowling that housed a Dayton Electric fan (Model 2Z846A, Moraine, OH), which pushed 26°C air from the chamber through a Dayton cartridge 5W921 filter into the tunnel. Laminar airflow (25 cm/sec) was verified by smoke plumes. A 21K BTU window air conditioner conditioned the chamber air. The humidity of the chamber air was maintained at 25–55% RH and cooled or heated as needed. Effluent air from the downwind end of the tunnel was vented into an exhaust hood. Overhead incandescent white lights over the tunnel provided illumination.

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Two upwind odorant sources were provided; an attractant alone and an attractant plus a repellent. Each odorant source was associated with a platform that permitted mosquitoes to fly up the tunnel, make a choice between feeding platforms, land, probe, and feed. The OIC system had to determine the number of insects crossing a defined reference plane, as well as where the insects crossed, with respect to the tunnel width.

To test the OIC imaging system, 20 mosquitoes (5–15 day-old female *Aedes aegypti* L.) contained in a 20 cm long × 6.59 cm diam plexiglass release tube fitted with a rotating door on one end and cheesecloth on the other were positioned at the downwind end of the tunnel. Once the imaging system was triggered, the release tube door was opened, and the mosquitoes were freed and stimulated to fly upwind by human breath delivered by a volunteer exhaling into a breathing tube positioned at the center-upwind end of the tunnel. As the air moved down the tunnel, it passed over the 2 sample sources (1 and 2) and carried the odorant plume from each sample toward the insects (Fig. 1). Twin upwind smoke sources at positions 1 and 2 verified that the plumes intersected and mixed approximately halfway down the tunnel. Therefore, the light sheet was positioned under the transparent floor one-third of the way down the tunnel. When an insect passed through the light sheet it created a time- and positional-stamped image. Experiments were replicated multiple times with samples 1 and 2 at alternate locations to eliminate any bias of insects favoring one side of the tunnel. In each experiment, fresh mosquitoes were released in the tunnel.

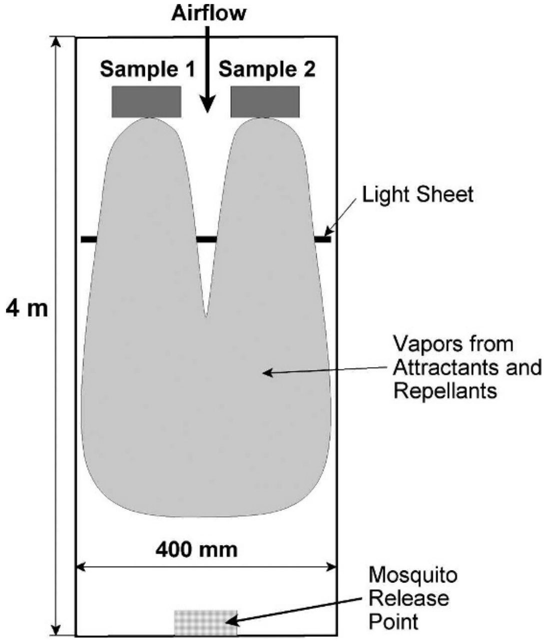


Fig. 1. Schematic representation of the wind tunnel with sample plumes simulated.

## Design constraints

An insect that flies between a light source and a digital line-scan camera creates a shadow on the CCD sensor of the camera. This image can be analyzed to determine the position of the insect relative to the light source. If the time that the image was taken is known, the insect then can be located both spatially and temporally along a line [394 mm × 0.192 mm (15.5 in. × 0.0076 in.)].

The system was constructed to fit around the flight tunnel. The camera and light source were located outside of the tunnel to avoid changing the air flow and odorant plumes. By placing 2 mirrors at 45° angles, the camera could be positioned adjacent to the tunnel and beneath the tunnel lights. To make the most effective use of the OIC, it was mounted on a rolling tripod that could be moved to different locations along the tunnel length while maintaining the camera alignment relative to the light source beneath the tunnel floor.

## Equipment

The following equipment was used in the OIC:

- Lightwise LW-SLIS-2048A-394 Linescan Camera (Imaging Solutions Group, Fairport, NY) with a line rate of 3,200 Hz
- Nikon 50 mm lens, 1.4 focal length, 54″ for field of view of 15.5, f8 (Nikon Inc., Melville, NY)
- Schott DCR III Light Source, 16″ Lightline fiber optic (Schott North America, Auburn, NY)
- PC (2.4 gHz minimum) with firewire Imagine Acquisition Board.

## Software

User interface software to control the camera and to perform the image analysis of the captured images was developed with LabVIEW 8.2.1 (National Instruments, Austin, TX). The camera was controlled by a separate driver software API, NI-IMAQ for IEEE 1394 cameras. Images were analyzed with NI Vision, an image-processing library that adds high-level machine vision and image processing to LabVIEW.

The program was made up of 2 while loops. A while loop is a computer code that allows 2 actions to occur simultaneously. The first loop captured images of insects and placed them in a queue. The second loop took the images out of the queue and analyzed them. Because the image

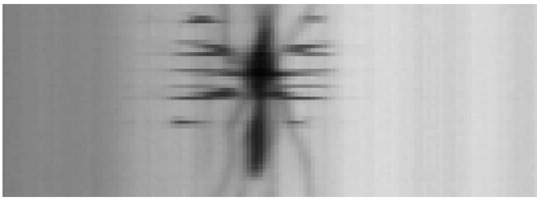

Fig. 2. Image of mosquito passing through the line sheet.

data were not passed directly, each loop ran in a separate thread at its own speed and did not slow down the other. The line-scan camera was configured to capture 2,048 × 16 pixel images in 8 bit monochrome at 200 frames per second. The image width was 390 mm (15.5 in.); therefore, each pixel of the image corresponded to 0.20 mm in width across the tunnel. The images were classified based on their minimum intensity value. A value less than a threshold indicated an insect was present. Images with insects were placed in a queue; all images without insects were discarded. This loop was repeated until the program was stopped.

The second loop ran while there were still images in the queue. If an insect spanned more than one image (i.e., 10 frames), they were stitched together to make a complete image of the insect. When the insect completely passed through the image plane, the final binary image of a large blob representing the insect's body and smaller blobs representing the legs was created (Fig. 2). A morphological erosion was applied to remove small blobs so they would not be counted. The center of mass was calculated for all insects in the image. The time and center of mass (i.e., pixel positional data) were written to a report file and the original image was saved.

## RESULTS

### Operation of the system

A control panel (Fig. 3) was constructed in LabView to facilitate operating the optical detection system. The user enters a unique name for a particular test, then clicks on the SETUP tab to view the image being captured by the camera and to set the imaging threshold. The threshold can be adjusted by varying the output of the light source until all of the pixels in the image have reached a threshold of over 50. With the 8-bit camera, each pixel can have a light intensity value from 0 to 255. Empirically, a triggering threshold at 50 was found to produce sharp images of the insects passing through the light sheet.

To start the experiment, the user clicks on the COUNT tab and then the Start button (Fig. 4). This initiates the timing and image capture routines described in the Software section. The complete image of the insect is displayed and updated as each insect is counted. The red stop button exits the program.

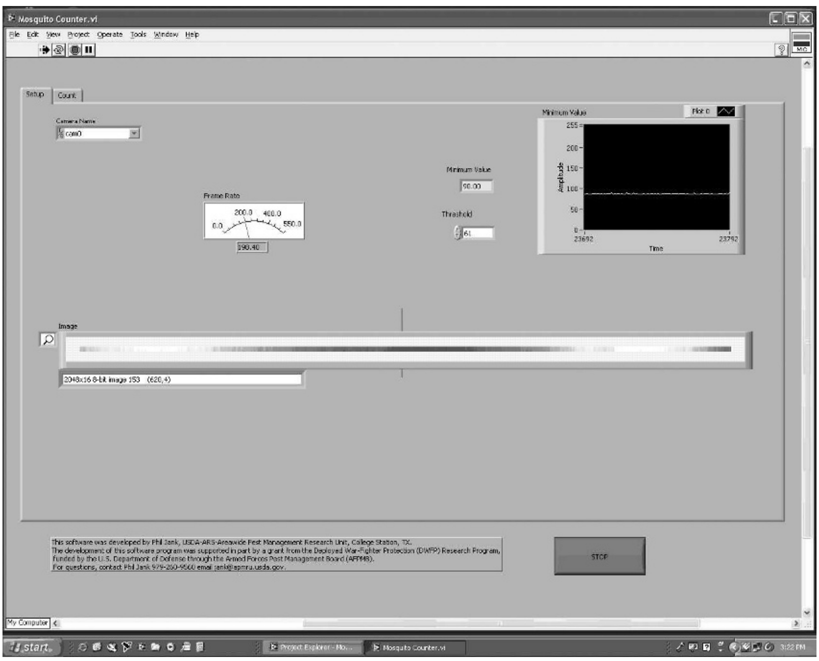


Fig. 3. SETUP control panel used to adjust the imaging threshold and triggering threshold before the start of a trial.

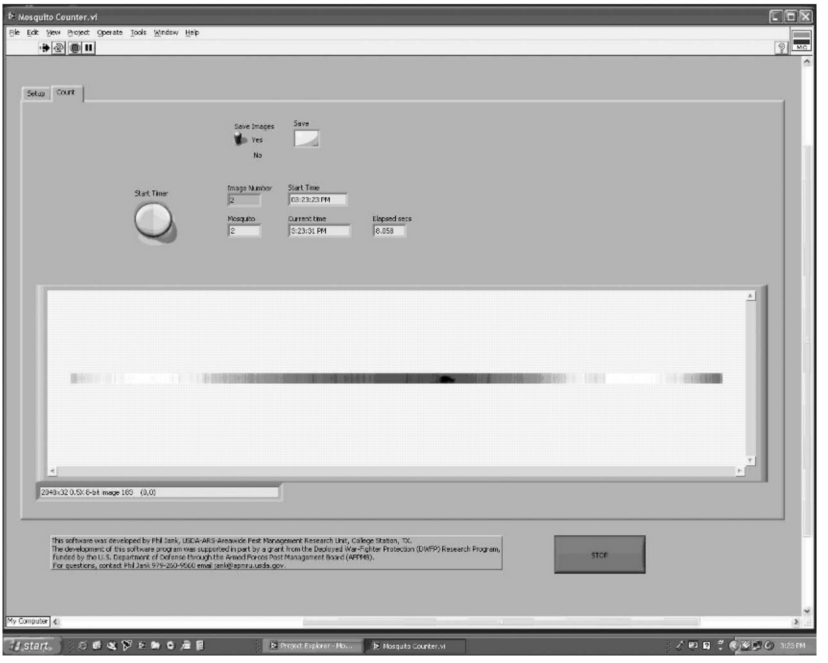


Fig. 4. COUNT control panel that starts and stops each trial and displays the images captured during a trial.

### Data processing

At the end of an experiment, a data file is created showing the count (how many insects passed through the light sheet), a time, and which pixel was initially triggered for each image. The data are then imported into a Microsoft Excel spreadsheet programmed with macros to automatically convert the pixel number into a distance (Table 1). The left edge of the tunnel is designated as 0 mm and the right edge as 390 mm. Therefore, the center of the tunnel corresponds to 195 mm. Using an IF statement, the routine calculates whether in the insect passed through the left or right side of the tunnel.

In the example given (Table 1), 19 insects were counted in 10 minutes. Twelve of the mosquitoes flew through the left side of the light sheet, and 7 flew on the right side. With multiple replications of the experiment, the resulting data can be used to determine if the insects prefer one sample source over another.

## DISCUSSION

The developed imaging system successfully counted insects and discriminated their location as they passed through a light sheet. The OIC system may allow researchers to investigate whether new and established materials attract or repel mosquitoes, as well as gauge the strength of the attractiveness or repellency. Although this work did not focus on developing the experimental protocol, it did show that our system could be used to support further protocol development and testing.

This system theoretically can also be used to calculate insect velocity. With a known frame rate and an insect image length, the time required for the insect to fly through the light sheet can be

Table 1. Test results showing 19 mosquitoes passing through the light sheet with positional and temporal data shown.

| Count | Time | Pixel | Location (mm) | Wind tunnel side (L or R) |
|---|---|---|---|---|
| 0 | 10:55:14 | | | |
| 1 | 10:55:25 | 544 | 104.4 | L |
| 2 | 10:55:32 | 44 | 8.4 | L |
| 3 | 10:55:55 | 672 | 129.0 | L |
| 4 | 10:56:04 | 1,978 | 379.8 | R |
| 5 | 10:56:59 | 1,237 | 237.5 | R |
| 6 | 10:58:09 | 1,087 | 208.7 | R |
| 7 | 10:58:26 | 619 | 118.8 | L |
| 8 | 10:59:05 | 1,321 | 253.6 | R |
| 9 | 10:59:07 | 1,047 | 201.0 | R |
| 10 | 10:59:08 | 840 | 161.3 | L |
| 11 | 10:59:08 | 295 | 56.6 | L |
| 12 | 10:59:08 | 301 | 57.8 | L |
| 13 | 10:59:27 | 1,101 | 211.4 | R |
| 14 | 10:59:55 | 596 | 114.4 | L |
| 15 | 11:01:09 | 380 | 73.0 | L |
| 16 | 11:01:26 | 1,646 | 316.0 | R |
| 17 | 11:02:00 | 361 | 69.3 | L |
| 18 | 11:04:03 | 158 | 30.3 | L |
| 19 | 11:04:51 | 972 | 186.6 | L |
| Total count on left side | | | | 12 |
| Total count on right side | | | | 7 |

calculated. Given the insect's length, the flight speed can be calculated. For example, with a frame rate of 200 frames per sec (0.005 sec per frame) and an image length of 40 frames, the time required for the insect to fly through the light sheet is 0.2 sec (40 frames $\times$ 0.005 frames per sec).

For a 5-mm-long insect, the flight speed would be 25 mm/sec (5 mm divided by 0.2 sec). The current system is not reliable because the legs and wings of an insect trigger the image capturing (Fig. 2). Images must be processed manually so that only the body of the insect is used to count the number of frames. Since mosquitoes are not the same length and do not all fly perpendicular to the light sheet, additional experiments are necessary.

A shortcoming of the system is that the direction of flight through the light sheet presently cannot be discriminated. Because the photo detector is a single pixel wide, the same image is created if the insect is flying upwind or downwind. This will be addressed in future iterations of the system by positioning 2 systems with the light sheets approximately 1–2 cm apart but with the light directions orthogonal to each other. One sheet will shine from the bottom to the top of the tunnel; the other will shine from left to right. This positioning should allow X and Y positional and directional data to be collected. For example, if system 1 triggered before system 2, one would know that the insect was moving upwind in the tunnel. The time differential between the 2 triggering events can also be used to calculate velocity if individual insects passing through the light sheets can be discriminated.

The OIC was successfully tested by a real-world application. The system integrated a line-scan camera and a light sheet along with data collection and image-processing software to determine both number of insect crossings as well as the location of each crossing. We hope that future configurations of the system provide reliable velocity as well as 2-dimensional positional data. The OIC provides a straightforward and reliable method for measuring and recording insect spatial and temporal information as they pass through an established plane.

## ACKNOWLEDGMENT

This study was supported in part by a grant from the Deployed War-Fighter Protection (DWFP) Research Program, funded by the U.S. Department of Defense through the Armed Forces Pest Management Board (AFPMB).

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