

Track3D: a new system for tracking, visualization and analysis of insect flight behavior in 3D

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Abstract: Wind tunnel systems are widely used in research on chemoreception and olfactory orientation of insects. Data collection often implies manual scoring of take-off and landing times, or video recording of flight in either the horizontal or vertical plane (2D), using a single camera. However, 3D tracking provides a more complete description and analysis of flight patterns than 2D coordinate data sets. With some species, 3D analysis is indispensable to understand their orientation in space and response to visual or olfactory stimuli.

Here we present a new video-based system for automated tracking of insects in a 3D space. The Track3D system records the flight of an insect in a test chamber or wind tunnel, visualizes the trajectory in 3D and calculates a large number of movement parameters. The system consists of tightly integrated hardware and software components. Insect flight is recorded using two synchronized video cameras and stored in high-resolution digital video files, using Media Recorder software running on a Windows 7 computer. From these files, the insect's movement is acquired by EthoVision XT video tracking software. The program records the 2D coordinates of the moving insect in each individual video image. Subsequently, the Track3D program – after 3D calibration using a specially designed calibration frame – converts the 2D coordinates from each camera view into one set of 3D coordinates. This track can be visualized in 3D, played back, rotated, and zoomed in/out. The software also calculates a large number of flight parameters, including distance moved, tortuosity, velocity (absolute and ground speed), heading and turn angles (relative to wind speed and different planes) for user-defined zones (odor plume and sectors of the wind tunnel).

The Track3D system has been validated in research on the behavior of the nocturnal malaria mosquito *Anopheles gambiae*. Responses to different human host cues involved in the foraging behavior of mosquitoes were studied by quantifying flight track characteristics in a wind tunnel. The insects were tracked while navigating through a plume of host-emitted cues under nocturnal conditions. Because of the low light intensity, infrared lighting and IR-sensitive CCD cameras were used. In the absence of host stimuli, flight paths were relatively short and flight speed remained nearly constant over the entire track. In contrast, exposure to human odor caused highly convoluted flights. Flight speed was greatest when mosquitoes were exposed to odor + heat. With these stimuli, flight speed decreased when the insects arrived near the source. This quantitative analysis of nocturnal host-seeking mosquitoes is a new step in the development of effective monitoring and preventive techniques for the control of malaria. The data show that a considerable proportion of flights take place in the vertical plane, demonstrating the value of 3D analysis of insect flight behavior.

Key Words: video tracking, wind tunnel, mosquito, flight behavior, EthoVision, Track3D