Track3D: Visualization and flight track analysis of *Anopheles gambiae s.s.* mosquitoes

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Female malaria mosquitoes locate their hosts by olfaction [1]. Little is known about nocturnal upwind navigation when the mosquito encounters host cues. This paper presents recently implemented techniques used to visualize and analyze the flight path of mosquitoes under low light conditions.

A wind tunnel equipped with a digital recording system was developed to study the flight behavior of *An. gambiae s.s.* in 3 dimensions (Figure 1). After initial tests [2], a new software package, "Track3D" [3] was developed as an add-on tool to Ethovision 3.1 to optimize automatic tracking. An air treatment system was developed and installed by Facility Services Tupola, Plant Sciences Group, WUR, The Netherlands, to ensure a constant flow of purified warm and humid 'tropical' air into the wind tunnel. Single mosquitoes were released in the wind tunnel and exposed to clean acclimatized air, human skin odours, either with or without a heat source.

The side walls and floor of the wind tunnel were constructed of black recycled polycarbonate and the ceiling was made of Lexan polycarbonate (WSV kunststoffen, Utrecht, The Netherlands). The dimensions of the flight arena are presented in Figure 1. Mosquitoes were made visible to the automatic tracking system by infrared lighting. Four infrared light units (Tracksys, Nottingham, UK) were placed at the front of the wind tunnel, facing the air flow. Each unit contained an array of 90 infrared LED's emitting light with peak output at 880 nm. To optimize lighting conditions four IR lights containing 168 LED's each (>920 nm) (Reinaert Electronics, Amsterdam, The Netherlands) were added in the same line as the Tracksys IR lights. The reflection of IR light from the mosquitoes' wings (1 wing ≈ 2.8mm) was filmed with cameras sensitive to infrared. Two Cohu 4722 monochrome CCD cameras with Fuji non-tv 9mm/f1.4 lenses were used. The cameras were attached to the ceiling of the experimental room such that their directions of view formed an angle of 40 degrees. Other camera orientations are possible, but this position minimized the reflections on the transparent top of the wind tunnel. Videos were recorded using Noldus MPEG Recorder 1.0 software (Noldus Information Technology, Wageningen, The Netherlands) that digitized the images coming from the cameras using an encoder board installed on the PC. The software saved video to a full D1 resolution, MPEG-4 video file, 704x576 pixels at 25 frames/s. The software ensured that recording started exactly at the same time for the two cameras, so the images stored in the two video files were synchronized. EthoVision 3.1 (Noldus Information Technology, Wageningen, The Netherlands) was used to analyze the video files. EthoVision 3.1 detected the mosquito on the dark background and stored the 2-D coordinates of its centre on a track file (one file for each camera).

3-D reconstruction of flight tracks

For a precise 3-D reconstruction of a mosquito flight, Track3D required input for lens correction, calibration, filtering and interpolation. Track3D accommodated the air velocity and odour plume as created for the experiments; it produced 3-D target coordinates, accuracy checks for calibration and a 3-D reconstruction. Twenty eight flight parameters were calculated and presented per mosquito track in Microsoft Excel (Microsoft Office Professional edition, 2003) output files. The target path was presented in a 3-D graph with the possibility of flight animation including different markers depending on the target position relative to the defined plume (Figure 2).

After positioning of the cameras above the wind tunnel, a calibration object of 60 x 58.5 x 57.5 cm was placed inside the flight arena. The object, made of black epoxy aluminum, had 28 markers distributed in two levels and with known 3-D coordinates. Markers on each calibration image (for each camera view) were selected in a fixed order, after which Track3D was used to determine the marker centroids. From the two sets of 2-D and known 3-D coordinates of the calibration object, Direct Linear Transformation (DLT) parameters were calculated. The calibration results were checked in two ways. First, the known 3-D coordinates were combined with the DLT parameters to calculate the expected 2-D marker coordinates. These are compared with the measured camera coordinates. Second, the 3-D coordinates of the calibration markers were calculated from the measured 2-D marker coordinates and the DLT parameters. They were compared with the known 3-D coordinates. The calibration accuracy of the used set-up was 0.5 % of the dimensions of the tracking arena.

To reduce noise the 2-D coordinates of tracked mosquitoes were smoothed by a Butterworth filter in combination with the zero phase shift routine filtfilt from Matlab 7.0 (Mathworks) with settings 'filter order 2' and cutoff frequency 8 Hz. Missing values, if not more than four at a row, were filled in by interpolated values. To this end, third order spline functions were used for all coordinates in a coherent block of data.

All flight parameter definitions are listed in the reference manual [3]. For the correct calculation in Track3D of flight parameters relative to air, the air velocity was set at 20 cm/s in the positive x direction. For each target position it was examined whether the mosquito flew inside a defined odour plume, a transition zone of 25 mm immediately outside the plume, or outside the transition zone. The odour plume was described as a cone. Its dimensions were estimated after all behavioral tests were completed by releasing smoke produced by a Safex® fog generator, F2010^{plus} (Safex-Chemie Gmbh, Schenefeld, Germany) using Safex® perfume free fog fluid. After this visualization process, the estimated position was defined by the apex, a point further on the axis, and the cone angle. Plume dimensions were estimated for treatments both with and without a heat source.

An. gambiae s.s females took off for upwind flight in 78 % of all cases tested (N=201). There was no difference in flight response between the four treatments ($P > 0.05$), however, mosquitoes exposed to the combination of odour and heat landed significantly more often at the source (47%, N=56) compared to the other treatments where landing at the source ranged between 0 and 7% ($P < 0.05$). Tracks were relatively short and in a straight line for the control treatment (e.g.

Figure 2A). The combination of human skin odours and heat elicited flights of longer duration with more crosswind behavior (e.g. Figure 2B).

Data will be presented to demonstrate the relevance of the system as a tool for the study of host orientation in mosquitoes.

Discussion and Conclusion

The automated tracking system successfully recorded and analysed flight behavior of nocturnal mosquitoes whose principal orientation cue for host recognition is smell [1]. The technical constraints associated with the recording of a fast flying small object, moving in 3 dimensions, were met resulting in a tool with which the behavioral parameters of mosquitoes and other insects can be accurately recorded and analysed. Track 3D is commercially available from Noldus Information Technology (www.noldus.com).

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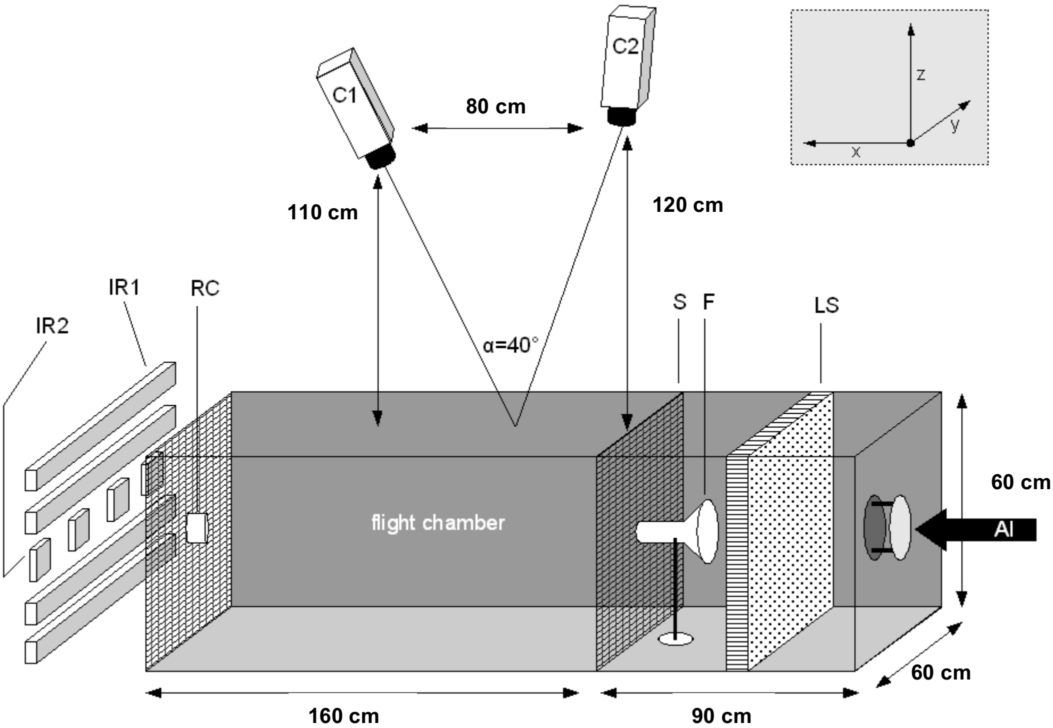

Figure 1. *Wind tunnel set-up. Air inlet (AI), lamination screen (LS), glass funnel containing heat element (F), mesh screen (S), release cup (RC), cameras (C1,2), IR lights type 1 (IR1), IR lights type 2(IR2). Mosquitoes were in view of both cameras up to 60 cm downwind from the mesh screen (S).*

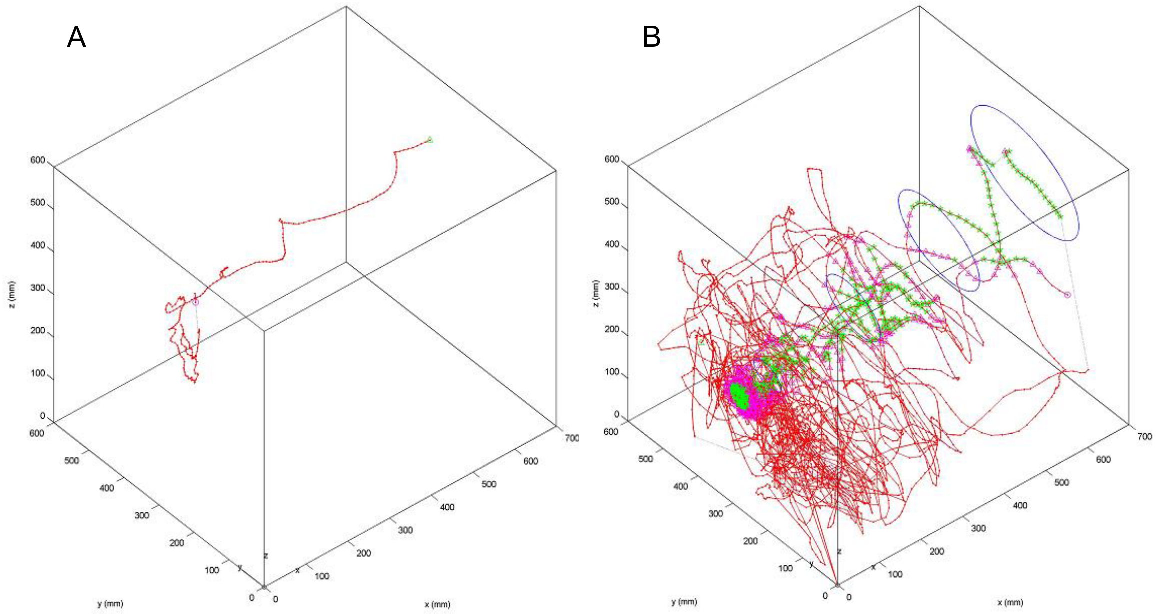

Figure 2. *2A shows a flight track of An. gambiae s.s. exposed to clean acclimatized air only. 2B shows a track of a mosquito flying upwind towards human skin odour in combination with a heat source. (Red) dots indicate samples outside the cone and buffer zone, (magenta) triangles are used for samples within the buffer zone and (green) stars indicate that the insect is tracked within the defined odour plume.*