

This article describes a new system for automatic recording and analysis of the flight behavior of insects in wind tunnels. It is the result of an intensive collaboration between Noldus Information Technology and the Department of Entomology, Wageningen Agricultural University. After years of design, development and testing, we are now approaching commercialization of the system as a standard instrument for entomological research. This article tells you how the system evolved from the drawing table to the laboratory and gives you an interesting look behind the screens of a complex development process.



[Willem Takken](#), tropical entomologist and scientific supervisor of the project, drew up the original specifications and coached the development process from the end-user's point of view.

[Piet Huisman](#), technician at the Entomology lab, constructed the wind tunnel setup, carried out the experiments and tested numerous prototypes.



[Marc Buma](#), system engineer at Noldus Information Technology, joined the project team in 1993 to design the software. Since then, he wrote thousands of lines of program code.

[Lucas Noldus](#), director of Noldus Information Technology, was in charge of functional design and overall project management.



HOW DO MALARIA MOSQUITOES FIND THEIR HOST?

Insect behavior is one of the key areas of interest at the Department of Entomology at Wageningen Agricultural University. Especially vision and olfaction of these tiny creatures, in relation to their orientation behavior, are subjects of ongoing research. One of the research models is the malaria mosquito, vector of a disease which continues to affect the health of millions of people in the tropics. Staff scientist dr. Willem Takken has been studying malaria mosquitoes for almost a decade. His research concerns the odors playing a role in the attraction of mosquitoes towards their (human) host. Unraveling these chemical signals is a prerequisite for the design of better repellents or trapping methods.

RECORDING FLIGHT TRACKS

For his research Takken uses wind tunnels to examine the flight behavior inside and outside an odor plume. Wind tunnels used by entomologists are usually rectangular tubes of 1-3 m long and 0,5-1 m diameter in which airflow, temperature and humidity can be controlled. Until recently, data collection was a 3-step process: a test was videotaped using an overhead camera; the track was replayed on a video monitor while the operator manually

traced it onto an acetate sheet, and this hardcopy was used to measure various parameters. This process was not only extremely time-consuming but also quite inaccurate, because measurement errors could easily accumulate.

ENTOMOLOGISTS AND COMPUTER ENGINEERS TEAM UP

Unsatisfied with the laborious manual methods, Takken looked for ways to automate the wind tunnel experiments. That was when, in 1991, he decided to consult his former colleague dr. Lucas Noldus about the development of an automatic video tracking system. At that time, Noldus was working on a tracking system for rodent studies, which would later become the EthoVision system as it is marketed today. However, while rats and mice stick to a 2-dimensional plane, the mosquitoes had to be tracked in a 3-dimensional space. In spite of many technical hurdles that would have to be taken, the development of a 3D tracking system seemed feasible. Takken and Noldus formed a team and set out to work.

WHY MOSQUITOES ARE HARD TO TRACK

The basic technique used in 3D video tracking is similar to that used in a 2D system. A scene is observed using a video camera. A series of images is digitized and stored in computer memory. The software then determines the location of the object of interest (i.e. the mosquito) in the image and stores its spatial coordinates. From these data, movement characteristics can be derived. The design of the 3D tracking system, however, confronted the team with a number of specific problems:

- Three-dimensional tracking requires simultaneous observation from two different viewpoints.
- Malaria mosquitoes are only active during dusk or at night.
- They fly in a very unpredictable pattern, with sudden zig-zag turns and a highly variable speed.
- The insects are very small: their thorax is only some 3 mm wide, while the minimum observation area was a wind tunnel segment of 60 x 60 x 60 cm, i.e. 200 times the size of the animal.

FLYING THROUGH THE NIGHT

The absence of flight during normal daylight and the mosquitoes' small size required a special combination of illumination and background, and CCD cameras with a high image resolution. Initial tests with low-intensity visible light and a white background failed because the contrasting part of the mosquito's body (the thorax) turned out to be too small to be reliably detected across the entire focal depth of the camera field. However, the use of infrared light and a black background did work: the reflection of the light on its transparent wings causes the mosquito to appear as a bright white spot in the video image.



*The set up for automatic recording and analysis of the flight behavior of insects in a wind tunnel.
(photo courtesy of Wageningen Agricultural University)*

AUTOMATED OFF-LINE DATA ACQUISITION

The highly variable flight speed required sampling at full video frame rate (i.e. 25 images/s). Because two cameras are used simultaneously, each second of observation results in 50 high-resolution images. Processing this massive load of data in real time is not yet possible with currently available (and affordable) PC-based imaging hardware. Therefore the team chose for off-line processing: observations are first recorded on video tape, and data acquisition is done automatically after a series of trials has been completed. An experiment management program maintains a database with independent variables (odor, insect number, etc.) and start/stop frames for each trial. Using a time code reader and tape controller, the system can automatically retrieve each trial from tape and extract the mosquito's position on a frame-by-frame basis. The pairs of 2D coordinates produced by the two cameras have to be translated into a single 3D coordinate, representing the exact position of the mosquito in the wind tunnel at each sample. This was a special challenge, requiring the use of advanced spatial calibration and reconstruction algorithms.

REPLAYING AND ANALYZING FLIGHT TRACKS

The last part of the project concerned the development of visualization and track analysis software. Flight tracks can be replayed in a 3D spatial representation and viewed under different angles and zoom ratios. One can thus inspect a track from a viewpoint unavailable in reality, e.g. seen from the odor source (where a filter normally obstructs your view). Tracks can also be plotted as 2D projections (upwind, crosswind and ground plane). The analysis program computes parameters such as traveled distance, linear or angular velocity, turn angle, flight heading, and the distribution of time over spatial zones. A zone of particular interest in wind tunnel studies is the odor plume. With the aid of special imaging routines which store the time-averaged 3D dimensions of the plume (visualized with contrasting smoke), the software can calculate the number of entries into the plume, the flight direction inside vs. outside the plume, etc. These statistics give the scientist a grip on the orientation of insects to olfactory cues.

A HAPPY LANDING!

After more than two years of development, a first prototype was installed at the Entomology laboratory in July 1994. Since then, continuous testing and refinement of the software have resulted in a stable product which our team is proud to present to the entomological research community. Willem Takken's group is using it every day - we hope that many labs will follow!