

VIDEO ANALYSIS IN BEE BIOLOGY USING NEURO INSPECTOR[®]

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INTRODUCTION

This paper describes *Neuro Inspector* - a simple system to detect and record animal behavior in real time. Our system can be used in organisms as diverse as the acellular paramecium to humans. In addition to its use in a wide range of animals, *Neuro Inspector* can be used to analyze a wide range of behavior including various types of locomotion, drinking, and more discrete behaviors such as movement of particular body parts or segments. This paper describes the state of the art image processing software Neuro Inspector and illustrates its usefulness in experiments on honey bees.

Many applications are available on the market to use computers along the instruments to automatically log experimental data or even make more complex experiment control (e.g. Lab View, National Instruments). Very few are available to do on-line analysis of video data. Most of that are oriented to the observation of specimen position (Noldus et al 2001, 2002, see links: Noldus, Vidmex,) and in some cases also shape and orientation of that specimen (Fry et al 2000, see link: Biobserve). Classical solution is animal tracking program. Before personal computers have become so powerful to perform grabbing of live video into computer, that was achieved by separate special processing. At that stage, PC was used just to make settings in processing unit and to collect processed data. With development of PCs, now it is possible to do live grabbing of full video sized images and to perform some analysis. Several solutions are available on the market. Most of them offer different kind of forms of animal tracking programs. In our studies we are interested along classical tracking also in monitoring of specific body part position and movements. Neuro Inspector is successfully used in industrial environment for real-time on-line inspection of numerous products. It has also been successfully applied in measurements in biology field. In our department has been successfully used to monitor position and movement of body parts of authentic cave salamander *Proteus anguinus*. We are reporting in this paper one unique solutions in honeybee behavior studies, which can be hardly achieved by other software. We configured several setups to measure antennae positions during trophallaxis and feeding of honeybees. Several other solutions are in development phase and will be reported during oral presentation at the Apimondia conference.

MATERIALS AND METHODS

We were performing different behavior experiments with honeybees reared at our Beekeeping Experimental Station at Biology Center in Ljubljana. Bees were authentic carniolans (*Apis mellifera carnica*, Pollman) reared in LR like hives based on Slovene AŽ standard frame size.

In our specific experiments reported here, we used exiting bees, which were taken into the laboratory. Bees were first immobilized on ice, hooked into hooked into shells, fed at libitum and then kept for 24 hours at room temperature. After one day bees were tested for feeding with stick wet of 50% sugar solution. After that we formed pairs of bees. One bee from a pair was fed with 10ul of the test sugar solution. We recorded bee behavior during feeding with digital camera (Cannon MV1). We used these recordings to prepare analysis of proboscis extension, drinking monitoring and antennae movements during feeding from capillary tube. After feeding fed bee was put in front of hungry bee from the pair and placed 1.5 to 2cm away to disable possibility of direct touch and food exchange for two minutes. In this part of experiment we monitored antennae movements as a reference activity compared to the activity in the next step of experiment, where we put bees together to enable direct touch with the antennae and food exchange. We made 10 minutes of recording and observed antennae movements and proboscis extension. For method development we used recordings of feeding from capillary and recordings of antennae movements during trophallaxis. To improve contrast in mouth part region, we painted part of the bee head with diluted white acryl color.

In the developing phase of applying new image analysis tool we used recorded digital video of the experiments. In the next step, digital records have been transferred into PC computer with Pinnacle DV500 card and accompanying software. Then, from the digital records, the sequence of JPG files were generated. Those files were further used for off-line analysis performed by the Neuro Inspector (NI). Until recently, we were not able to perform on-line analysis due to the missing hardware. We discuss that and other possibilities in the Results and discussion section.

RESULTS AND DISCUSSION

We found out several possibilities for detection of antennae positions. The best solution would be to report where tips of antennae are directed. On the other hand is also important how antennae are stretched toward partner bee. The latest can be describe with two angles, first one can describe position around the head and second one deflection of antennae flabellum from that direction. We explore simple measurement of angle in radial profile based on position and size of rectangular window called "P-Window".

First we did some preprocessing with gray level transformation (Fig 1). We added two P-windows to clear some dark background. In the next step we defined two more P-windows on original frame and copied and rotated those contents in new image into two P-windows of the same size (Fig. 2). There we calculated center of the head based on starting image in analysis folder. We used coordinates in result table of NI to calculate relative position of analyzing P-windows to detect angles of antennae crossing at the side of the head (Fig. 3). The results were stored in a history file of NI, in a format compatible with MS Excel.

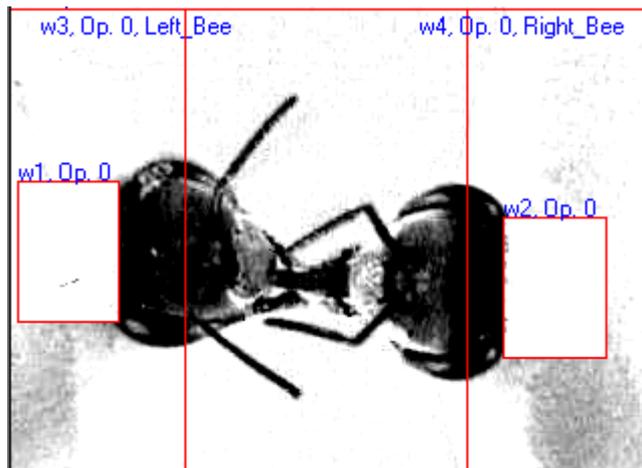


Figure 1: Preprocessed image of trophallaxis between two bees. Two P-windows (w1,w2) were applied to mask dark background. Two additional P-windows (w3, w4) were defined for further image processing.

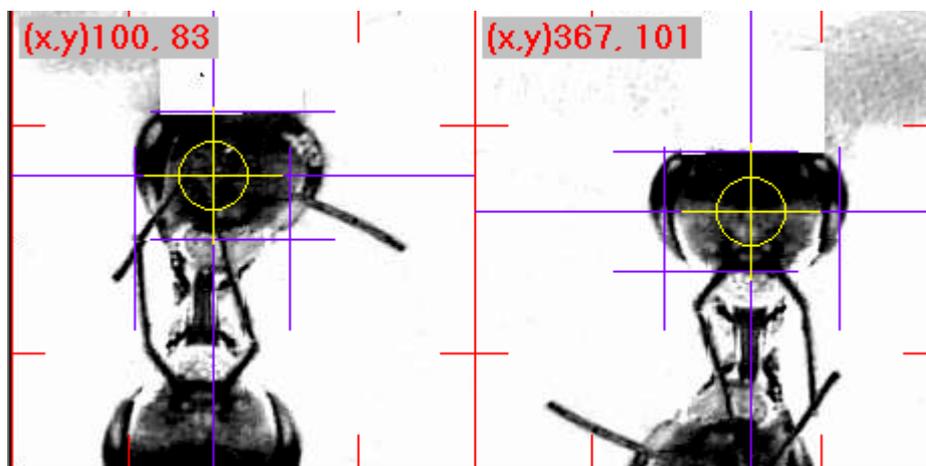


Figure 2: Center detection (circles with crosses) for both bees for relative positioning of analysis windows (see Fig. 3)

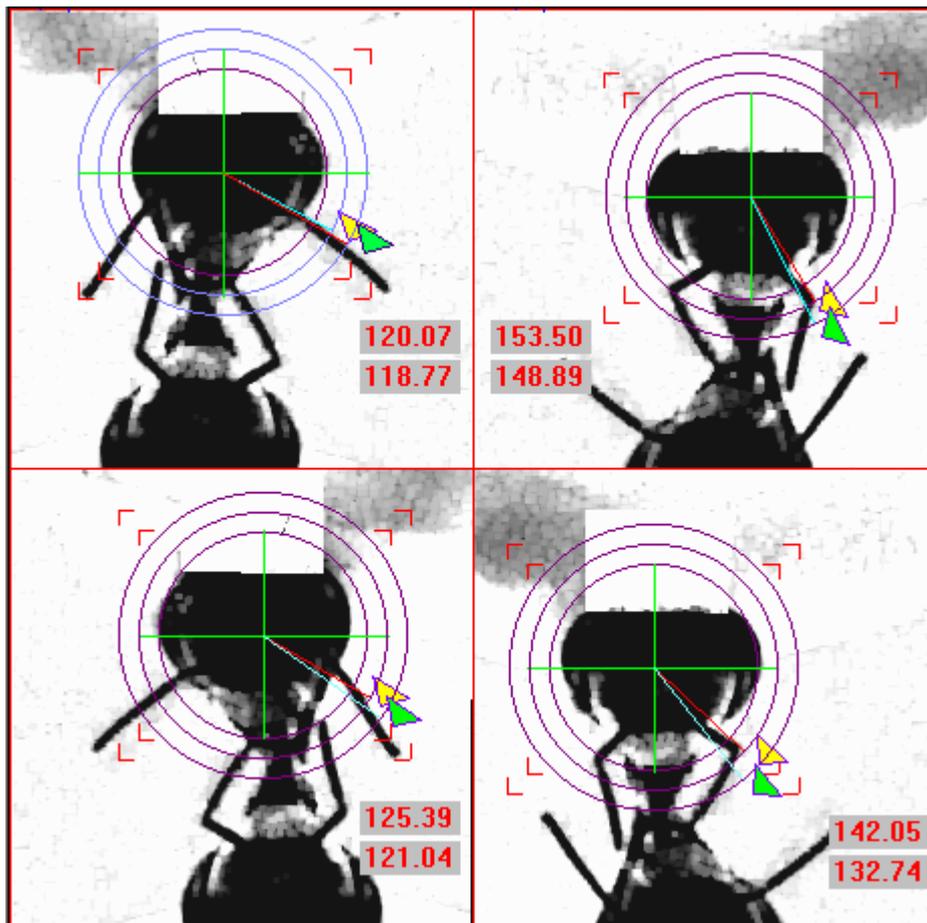


Figure 3: Detection of angles around the head where are stretched antennae. For each antennae a separate window was prepared to use simple possibility for angle detection from radial profile inside P-windows (w13-w20). These windows were positioned based on calculations in result table of the NeuroInspector using results from Fig. 2.

Recordings were not perfect as well some bees move one antenna also upward and we lost detection of such positions. During of-line analysis it is possible to detect such fails of automatic measurements. For that purpose we run NI with two copies. In one we went through analysis of images through time table settings with option to break run at each imported image. When we noticed failure of measurement we checked index in data file with previous obtained results (from history file) and reload that image and second opened NI program and manually measured angle with measurement tool. Based on obtained measurement we corrected data in Excel data file.

From the angle difference and radius for both radial profiles we calculated declination of the antenna flabellum from the direction when it crosses margin of the head. Crossing angles of all four antennae are shown in time course at Fig. 4. It can be seen that most active was right antennae of the donor bee. Such time plots can be used to explore mutual movements. Of course, there are also other possibilities to show antennae position. On Fig. 5 we compare crossing angle with declination of antennae flabellum. That is good to see some special outliers, since it is possible to make some mistakes during manual typing of angles in data sheet.

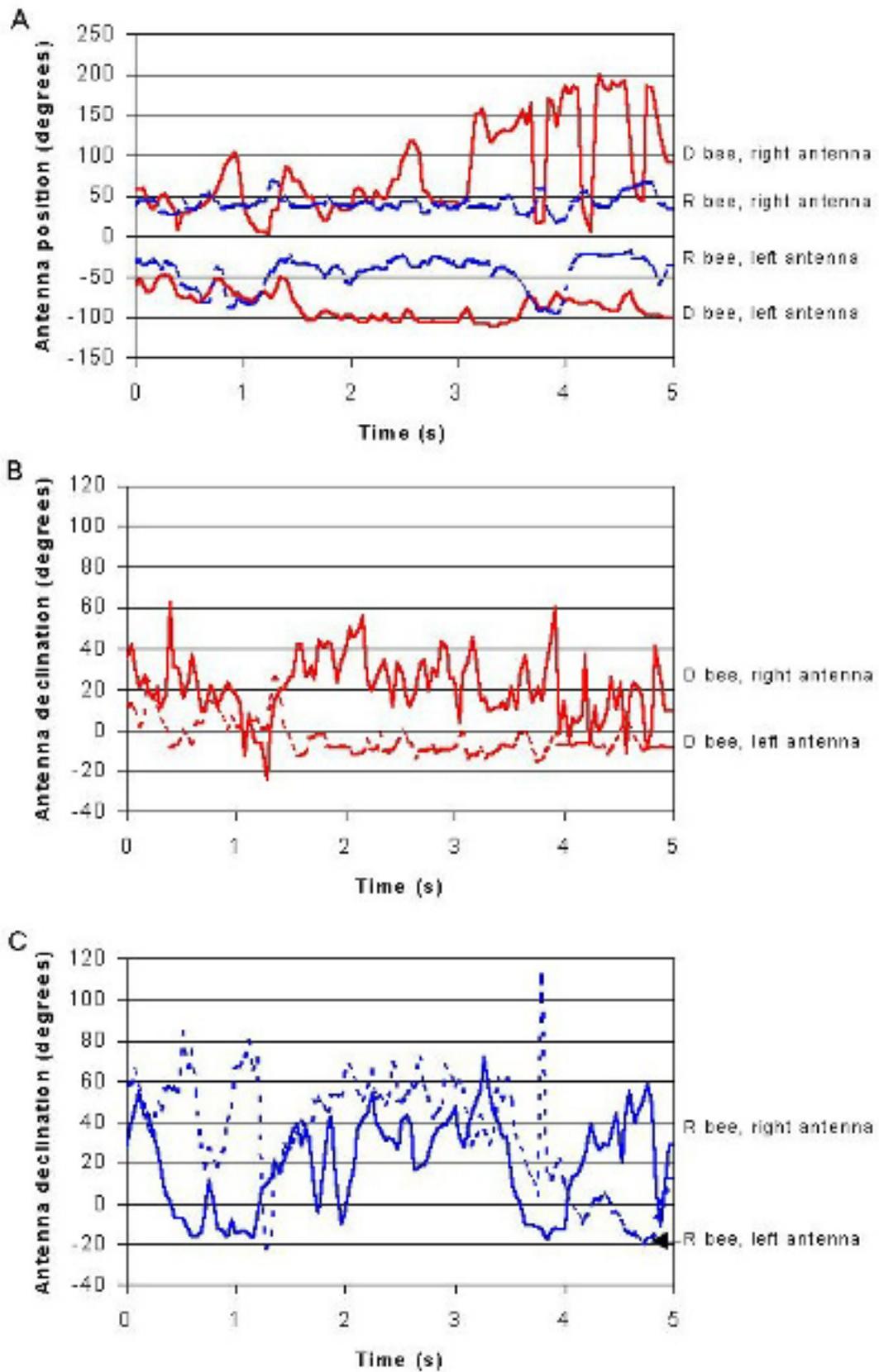


Figure 4: time course of angular positions and declinations of donor (d) and receiver (r) bees' antennae. Donor bee moved away right antenna from receiver bee to the end of trophallaxis (a). Receiver bee showed larger declination of both antennae toward mouth parts then donor bee (c and d).

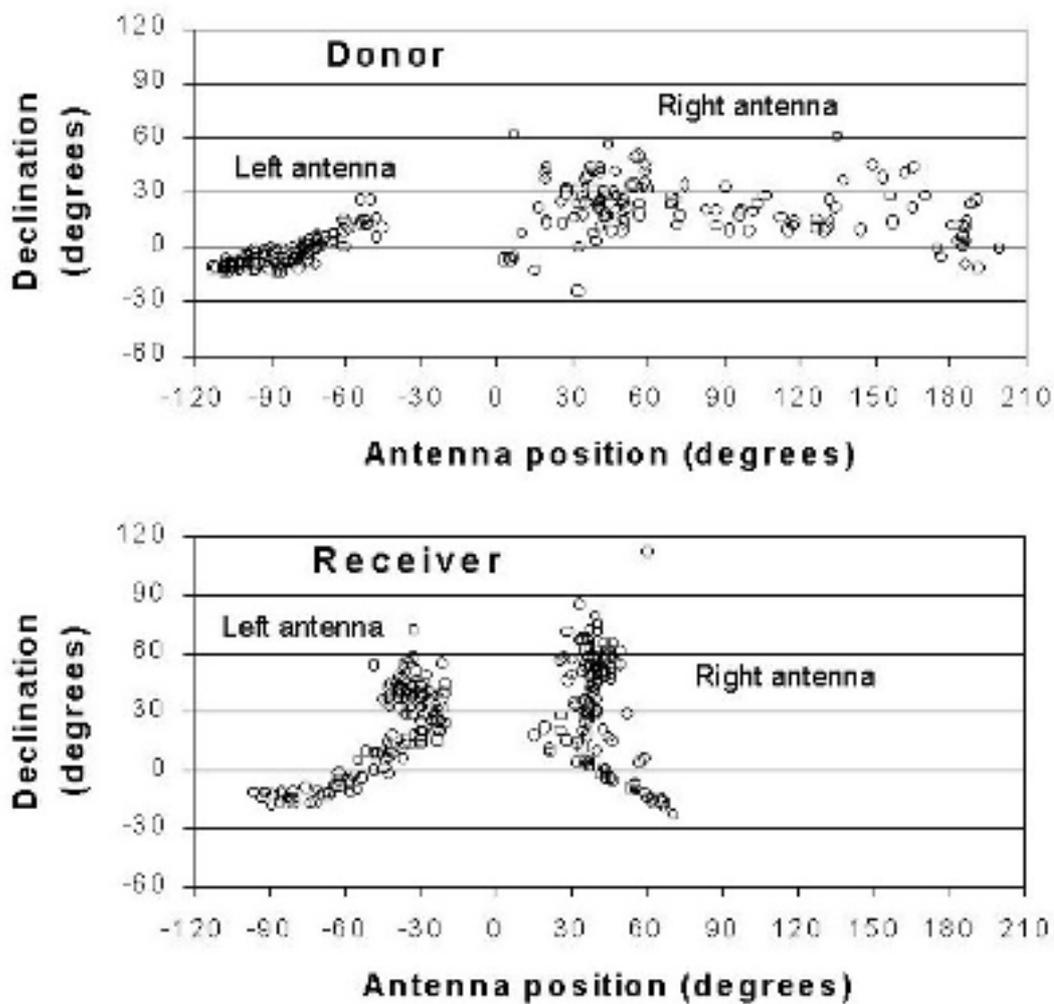


Figure 5: Correlation of declination to angular position of the antennae of donor and receiver bee. Same data are represented as on Fig. 4.

This described solution doesn't need special programming with Visual Basic for Applications (VBA). We can explore possibilities of NI environment and data manipulation software like MS Excel.

If we are just interested in antennae position around the head then we can obtain image presentation of that through combining differences between two images in a sequence through whole set of images. With such procedure we obtained Fig. 6. For easier presentation we can read radial profile as it is shown on the Fig. 6. We used simple VBA code to read profile in text file and plotted values in Excel (Fig. 7). With this representation could be problem when we get very frequent positions at the same spot. In that case we got a maximum possible value in the distribution (255 – level of grays). That was not the case in our example.

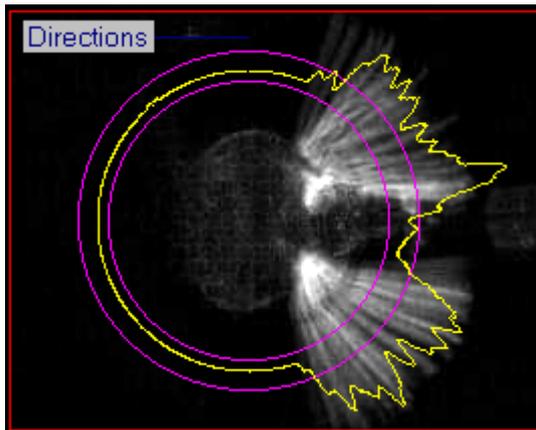


Figure 6: Radial profile through summary image of antennae positions during drinking from capillary. 125 single frames are combined in this image (see Results and Discussion).

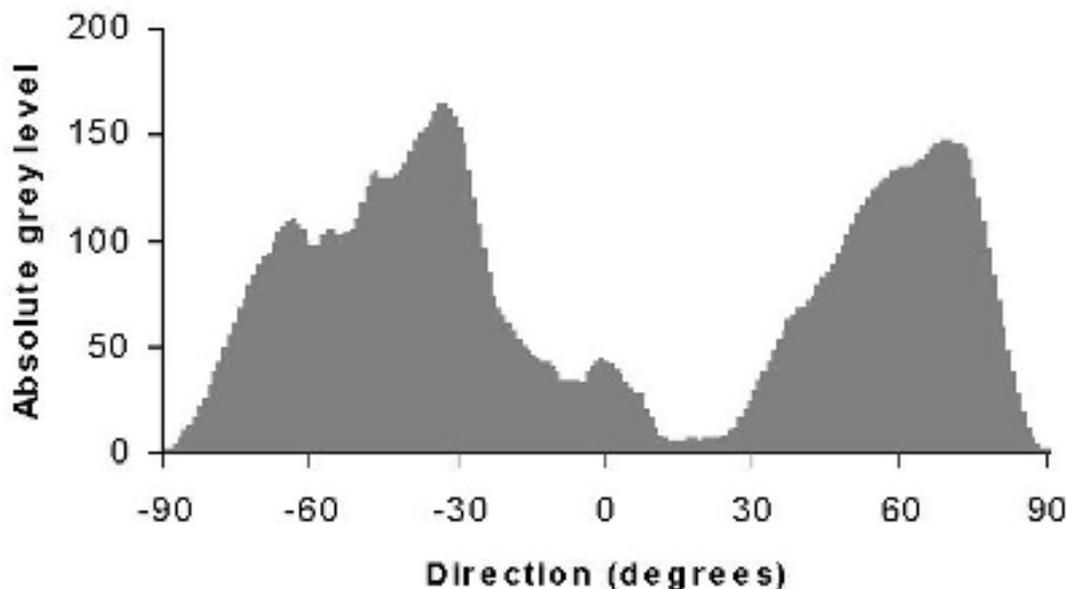


Figure 7: Histogram of absolute gray levels in the radial profile as detected in Fig. 6

There are also other possibilities to detect angle position. We tried to implement fiber model on preprocessed image (Fig. 8) and on subtracted image obtained from two consecutive frames (Fig. 9). Both approaches can be used, but we need to do more VBA programming to get angles from lines and, more important, to check possible overlapping of two opposite antennae. Sometimes also simple crossing causes problems with correct antennae detection. This is not a problem if we analyze only one bee in observation field, but that is not easy task for trophalaxis studies. We will further explore that possibility and report at the congress.

Neuro Inspector also enables simple tracking of several animals as can we do with other programs (see links Biobserve, Noldus, Vidmex). Along that it is possible to prepare procedures for identification of bees based on special codes on the thorax. We are exploring these possibilities and preparing solutions for future studies of social interactions inside cages or even in observation hive.

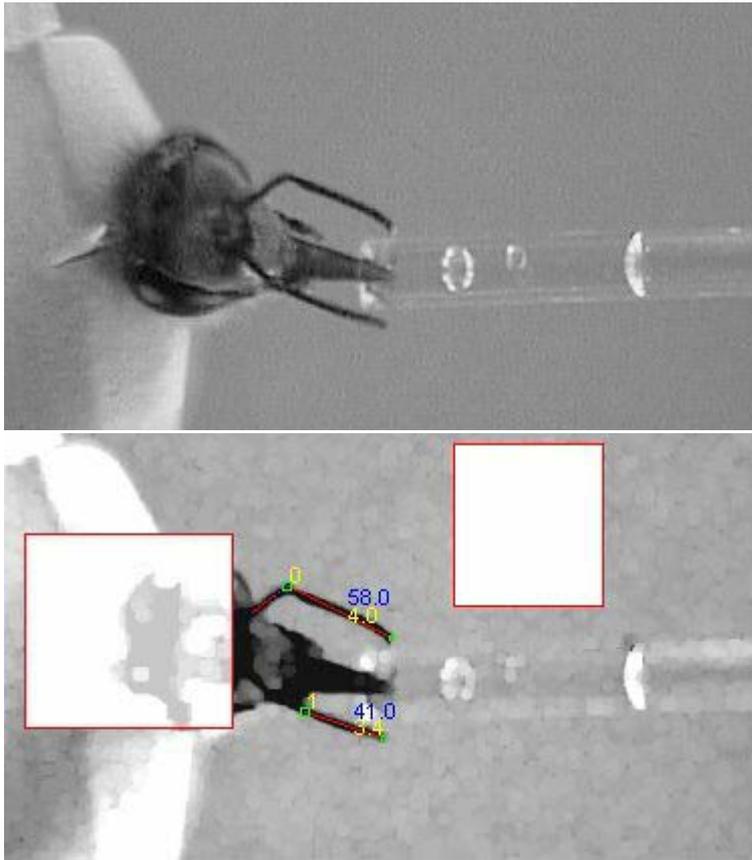


Figure 8: Application of fiber model for detection of antennae based on preprocessed image of drinking bee.

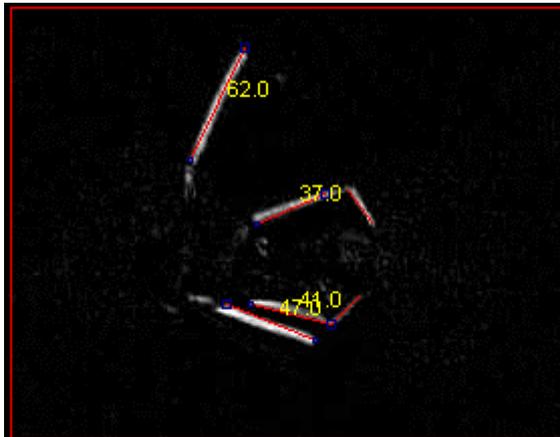


Figure 9: Detection of antennae with fiber model in trophallaxis experiment. Two consecutive images were subtracted to obtain image for analysis.

ACKNOWLEDGMENTS:

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Noldus LPJJ, Spink AJ, Tegelenbosch RAJ (2001) EthoVision: a versatile video tracking system for automation of behavioral experiments. *Beh Res Meth, Inst & Comput* 33:398-414

Noldus LPJJ, Spink AJ, Tegelenbosch RAJ (2002) Computerised video tracking, movement analysis and behaviour recognition in insects. *Computers and electronics in agriculture* 35 (2-3): 201-227

LINKS ON THE INTERNET

(1) Biobserve, <http://www.biobserve.com>

(2) LabView, National Instruments, <http://www.labview.com>

(3) Neuroinspector, <http://www.fdsresearch.si>

(4) Noldus, Ethovision,
http://www.noldus.com/products/ethovision/ev30/ev30_start.html

(5) Vidmex, Columbus Instruments, <http://www.colinst.com/brief.php?i=6>