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Automated tracking and analysis of behavior in restrained insects

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HIGHLIGHTS

- We present an algorithm for tracking the movement body parts of restrained animals.
- The tracking algorithm works with low frame-rate videos.
- The tracking algorithm automatically segments and tracks multiple body parts.
- We demonstrate the power of the algorithm in analysing insect behaviour.

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ABSTRACT

Background: Insect behavior is often monitored by human observers and measured in the form of binary responses. This procedure is time costly and does not allow a fine graded measurement of behavioral performance in individual animals. To overcome this limitation, we have developed a computer vision system which allows the automated tracking of body parts of restrained insects.
New method: Our system crops a continuous video into separate shots with a static background. It then segments out the insect's head and preprocesses the detected moving objects to exclude detection errors. A Bayesian-based algorithm is proposed to identify the trajectory of each body part.
Results: We demonstrate the application of this novel tracking algorithm by monitoring movements of the mouthparts and antennae of honey bees and ants, and demonstrate its suitability for analyzing the behavioral performance of individual bees using a common associative learning paradigm.
Comparison with existing methods: Our tracking system differs from existing systems in that it does not require each video to be labeled manually and is capable of tracking insects' body parts even when working with low frame-rate videos. Our system can be generalized for other insect tracking applications.
Conclusions: Our system paves the ground for fully automated monitoring of the behavior of restrained insects and accounts for individual variations in graded behavior.

1. Introduction

Insects are often used to study the neuronal mechanisms that underly behaviors ranging from sleep to higher-order associative learning (Sauer et al., 2003; Matsumoto et al., 2012; Menzel, 2012). When controlled stimulus conditions are needed, insects are often restrained and their behavior is monitored as movements of body parts such as their antenna or mouthparts. Insect behavior is often measured by human observers and recorded in the form of binary responses to prevent the introduction of subjective biases by the observer. This procedure is time consuming and it does not allow a fine graded measure of behavioral performance in individual animals.

In neuroscience the honey bee is a particularly powerful model animal for learning and memory research (Menzel, 2012). Associative learning of individual, fixed bees can easily be studied by classical conditioning, where an odorant is paired with a sugar reward. Whether a bee has learned the association is usually assessed by its proboscis (i.e. the mouthpart of the bee) extension response (binary all-or-nothing measure) (Bitterman et al., 1983). A bee extends the proboscis reflexively when stimulated with sugar

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water or with a previously conditioned odorant. Up to now learning and memory have been mainly assessed by a crude all-or-nothing measure (whether a bee reacts to a learned stimulus, or not). This binary measurement is not suited to reveal individual differences in learning and memory performance, for this purpose a graded performance measurement is required (Pamir et al., 2014).

A graded measure for learning and memory can be extracted from the temporal characteristic of the proboscis extension response, which contains information about whether a bee has learned an association or not (Rehder, 1987; Smith et al., 1991; Gil et al., 2009). Moreover, temporal patterns of antennae movement change upon sensary stimulation (Erber et al., 1993) and reveal internal states such as sleep and wakefulness (Hussaini et al., 2009; Sauer et al., 2003). To precisely analyze such dynamic behavioral monitors, tracking systems are required. However, available insect tracking systems often have the weakness that they require prior marking of the animal (Hussaini et al., 2009), and are often capable only of tracking single insects (Veeraraghavan et al., 2008; Landgraf and Rojas, 2007), working with slowly-moving insects only (Balch et al., 2001; Ying, 2004), or can track only one type of body part, i.e. bee's antennae (Hussaini et al., 2009; Mujagić et al., 2011).

We addressed this issue and developed a computer vision system which allows the automated tracking of the body parts of restrained insects while providing quantitative information about the movements of their mouthparts and antennae. This system can easily be adopted to other insects, and it allows one to implement novel approaches to analyze insect behavior using graded measures of behavioral performance.

2. Materials and methods

We will elaborate our system as follows. We firstly perform moving object detection by subtracting the static background (Section 2.3). The moving object detector generates a set of bounding boxes (BBs), which are rectangles that bound detected objects. We then preprocess the input frame to reduce undesired BBs including false, missing, splitted and merged ones (Section 2.4). The appearance model is constructed in Section 2.5. Finally we propose a tracking algorithm in Section 2.6, which is able to identify the label of each of the five moving objects: “1” for right antenna, “2” for right mandible, “3” for proboscis, “4” for left antenna and “5” for left mandible as shown in Fig. 1c. For the sake of clarity, in Table 1 we list all abbreviations and notations used in the paper.

2.1. Video acquisition

Honey bee foragers (*Apis mellifera*) were caught from outdoor hives and prepared as described in Szyszka et al. (2011). Small ant workers (*Camponotus floridanus*) were provided by C.J. Kleineidam. Colonies were reared in a climate chamber at 50–60% relative humidity and 26 °C. The founding queens were collected by A. Endler and S. Diedering in Florida Keys (USA). The ant's neck was pushed through a slit in plastic foil, and its head was fixed dorsally to the plastic foil with a low temperature melting, equal-weight mixture of dental wax (Deiberit 502; Dr. Böhme und Schöps Dental), and n-eicosan and myristic acid (both Sigma–Aldrich). Each individual insect was imaged at 30 frames per second using a CCD camera (“FMVU-03MTM/C” Point grey, Richmon, Canda) in order to record the head with proboscis, mandibles and antennae. The setup of the bee experiment is shown in Fig. 1a. Insects were recorded with or without odor stimulation and sugar feeding. Odor stimulus delivery was monitored by lighting an LED within the field of view of the camera, so that data analysis can be done relative to stimulus delivery. Insects were harnessed on a platform, with their head in fixed positions, but able to move antennae and mouthparts freely. The camera was set on top of an individual insect. The camera was fixed, and the platform to which the insects were fixed was moved when changing to a new insect for recording. Unlike the high speed camera used in (Voigts et al., 2008), which is capable of capturing videos at 500 frames/s, the frame-rate of the acquired movies in this paper was only 30 frames/s. Although it would be possible to record with a high speed camera, we aim at developing a system that uses affordable cameras such as web-cam or consumer level cameras and keeps the data volume low. Each video was about 30 min long and consists of 12 trials, with 16 individual honey bees each. For each trial, a single video to be processed was approximately 10–30 s long and had a frame size of 480×640 pixels.

2.2. Coordinate system setup

To extract the information of the relative position of each object to the insect head, it is required to set up the coordinate system. As the platform is not static during the changing of insects, the scene change is detected to ensure a static background before the actual tracking procedure starts. For scene change detection, the edges in each frame were detected using a Sobel Filter. The mean of all the blocks within the edge image is computed and compared to the mean of all the blocks of the previous frame. If the absolute difference of means between two blocks in consecutive frames is greater than a predefined value, the block is assumed to be changed. The scene is detected to be changed if the number of changed blocks is greater than a predefined number. The video is cropped into several shots automatically according to the scene change detection.

For each shot, the mean of the first ten frames is used to estimate the insect head's position. After thresholding, a dark region with the greatest circularity value and an area within the range of 0.33–2.6% of the whole image is selected as the segmented head, and the position of the origin is estimated as the left-most point of the segmented head (as shown in Fig. 1b). With the origin (marked as point “o”) and the centroid of the head (marked as point “c”) estimated, a new coordinate system is established by using the mandible as the origin, line “oc” as x-axis and the line orthogonal to “oc” as y-axis.

2.3. Object detection

For detecting moving objects, Gaussian Mixture Model (GMM) background modelling (KaewTraKulPong and Bowden, 2002) is used. The first five frames of each shot are used for training the initial model parameters of the GMM background model. As in KaewTraKulPong and Bowden (2002), background subtraction is performed by marking a pixel as a foreground pixel if it is more than 2.5 standard deviations away from any of the distributions of the background model. The background model is updated for each frame; and a static object staying long enough will be determined as part of the background. The model is suitable for our case, where a static background exists in each shot.

2.3.1. LED and sugar stick detection

As the LED is used to indicate when the odor is released, detection of the LED is part of our task. Due to the nature of the GMM background model, the detection of the LED fails when it is on for a few seconds. To address this problem, we store the BB of the LED when it is detected for the first time, and measure the intensity within the BB. If the intensity is greater than the average of the image, the LED is determined to be on.

The time when the sugar stick touches the insect is required for assessing the latency of its proboscis extension response. A BB that is attached to the dilated head having a width or height greater than 100 pixels is assumed to be the sugar stick.