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Behavior Analysis of Ants from Video Sequences

Abstract—The movement of small animals in well-defined environments is increasingly studied in many areas; including ecotoxicology, learning, and behavioural ecology. Here we describe an algorithm designed to analyze individual foraging ants from a colony of *Lasius niger*. The inputs to the algorithm were images from a video sequence. The algorithm performed a series of pre-processing steps to identify the ants from the pixels, measurements were extracted and individual ants were tracked in time. The location of the ants in position and time were recorded as heat maps denoting the favorite locations of the ants. The ants were videoed in a foraging experiment on a T-maze a single trail bifurcation.

Keywords—*ants; route learning; pheromone; collective decision making; foraging; segmentation; tracking; animal behavior.*

I. INTRODUCTION

There is great interest in the observation of animals in enclosed environments. Social insects such as ants can be observed due to their nesting habits, and combined with different environments; these can be used in experiments of foraging [1], decision making [2], [3] or impact of pollution [4]. Studies of animals in well-defined environments are not restricted to ants. Other animals like crustaceans, are used in experiments of ecotoxicology to test short or long-term exposure to contaminants such as insecticides [5] or nanoparticles [6]. Bees are also used to study foraging [7] or colony health [8], and these are of great importance to agriculture.

In the case of ants, there is interest in the observation of the movement of the individuals. Trail pheromones have been understood to be used as guides of movements between points. However, recent research has indicated that these trails are also used to regulate colony foraging and behavior through negative and positive feedback processes and can be complemented with individual memory [9]–[11].

Video is increasingly being used to record the foraging behavior of ant colonies. These video clips allow the visualization of the whole experiment and the dynamic nature of the experiments. However, the acquisition of the data is only the first stage of the analysis process. In many cases, data are acquired at a faster rate than can easily be processed "by

Fig. 1: Ant experiment setup. A *Lasius niger* ant colony was trained to collect 1M sucrose solution at the end of a T-maze.

hand". In this context, segmentation is the identification of the pixels that correspond to a single observed animal from a connected region or "blob". In addition, tracking is understood as following a single observed animal between consecutive time frames. Our group and others are attempting to improve the analysis of video data from on small animals. Preliminary work of segmentation, tracking and analysis of crustaceans in microfluidic environments [12], based on analysis of neutrophils in zebrafish [13], has shown great benefits in the automatic analysis of videos. Here we describe an algorithm to analyze the movement of ants that are foraging for food. The algorithm is fully automatic. It segments ants from the background with a few pre-processing steps. Individual ants can then be tracked and their overall movement quantified as heat maps.

II. MATERIALS AND METHODS

A. Study Species

We studied one colony of *L. niger* ants which were collected on the University of Sussex campus (U.K.). The colony had ca. 1,000 workers. The colony was kept in a plastic box (40 x 30 cm and 20 cm high) containing a wooden nestbox (15
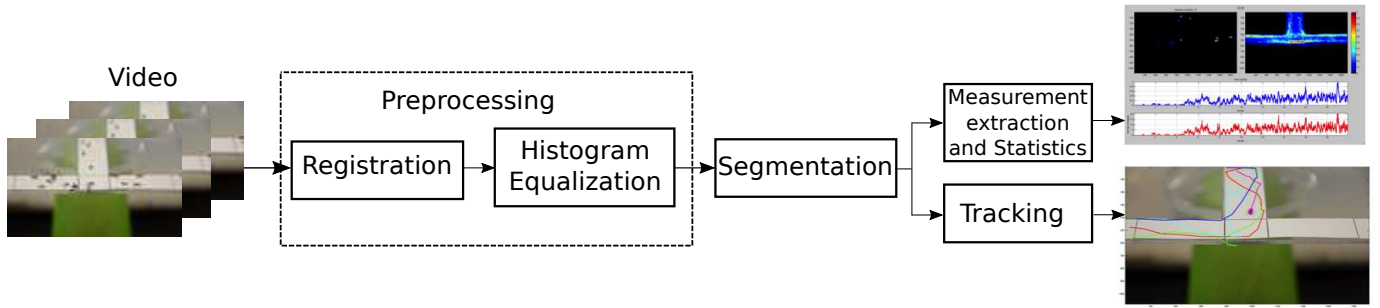
Fig. 2: Pipeline of the ant tracking system.

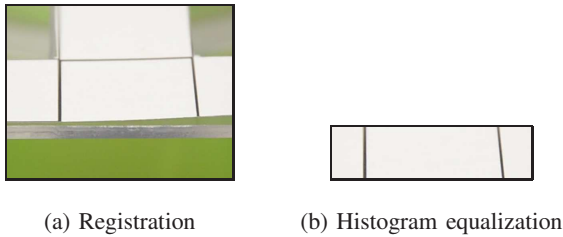(a) Registration (b) Histogram equalization

Fig. 3: Preprocessing references areas extracted from the first frame.

x 15 x 2cm high). The bottom of each plastic box was covered with a layer of plaster of Paris. The colony was starved for 5 days prior to the experiment to ensure that foragers were motivated to collect a 1M sucrose solution. For testing, the plastic box was connected to a T-shaped foraging trail. The stem of the trail was 15 cm long and each branch was 11 cm long (see [3] for details).

B. Experimental Procedure

The movement of the colony was tested using a single bifurcation procedure, similar to those described in [2]. The maze was covered with white printer paper. At the ends of the two branches were feeders of 1M sucrose syrup (Fig. 1). Video sequences were acquired with a static camera capturing 23.98 frames per second with 1920 x 1080 resolution.

C. Video Analysis

We developed a framework to analyze ant behavior that tracks individual ants and extracts measurements and statistics from the video sequence of a foraging experiment. Fig. 2 shows the pipeline, or sequence of processing steps, of the framework. The first task in the pipeline was to equalize its greyscale histograms with respect to the first frame of the sequence. In addition, registration of each frame against the original frames was performed to discard any slight movement of the bridge of the experiment. This step compensated for any variations in illumination and position during the experiment. Illumination correction is particularly important as conditions were not constant. In the second step, the frames were converted to greyscale and the foreground pixels, which correspond to the ants, were segmented by intensity. In the last two steps, the tracking and measurement extraction were performed by allocating each segmented blobs as one ant. No

attempt to segment the occasional case where two ants were joined in a single blob was made. The last step of the pipeline was the extraction of positional and temporal information from the segmented ants. The following subsections provide specific details on each task and discuss some presented issues.

1) Preprocessing: The objective of this task was to correct three main issues from the original video sequence. The video of the experiment was captured considering that is was meant to be analyzed manually by an expert and thus the illumination conditions were not ideal. First, the interaction of the observer caused illumination changes throughout the sequence and also the hands appeared in a few frames. Second, the T-junction bridge was not a solid structure and it moved horizontally and vertically across the video frames. Third, due to the camera lens and the distance to the bridge, the front of the bridge was in focus, whilst the back, closer to the nest, was blurry.

Registration. In order to save computation time, we selected a small reference area across all frames to measure the displacements rather than the full image. Specifically, the selected area corresponds to the T-junction union of the maze, as depicted in Fig. 3a. This area clearly shows horizontal and vertical changes. Additionally, this area was converted to its greyscale values.

Starting from the second frame, we matched the scale-invariant features (SIFT) [14] features of the reference area of each frame i with respect to the ones of the previous frame i-1. Both operations were done using the VLFeat library [15]. Then we calculated the vertical and horizontal displacements of frame i for the first n pairs of top match points as

$$\Delta x_i = \frac{\sum_{l=1}^{n} x_{i,l} - x_{i-1,l}}{n} \tag{1}$$

and

$$\Delta y_i = \frac{\sum_{l=1}^{n} y_{i,l} - y_{i-1,l}}{n} \tag{2}$$

respectively, where $(x_{i,l}, y_{i,l})$ correspond to the coordinates of the point l from frame i, and $n = 15$. Then we simple translate the pixels of the original image and its greyscale version.

Histogram equalization. This subtask was performed on the greyscale version of the frames. In the same manner as the registration subtask, we employed a small reference area that appears in Fig. 3b. Although this is a small area, it is representative of the changes in illumination as it covers a high activity pixel region.
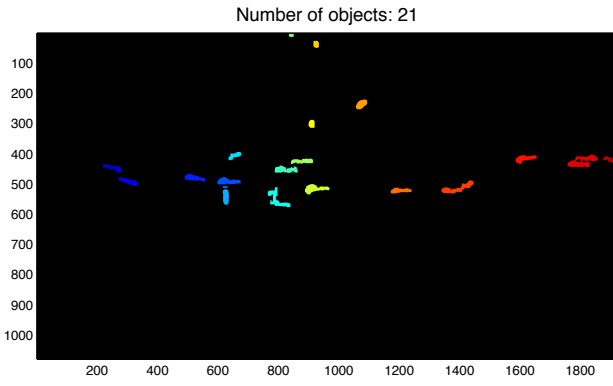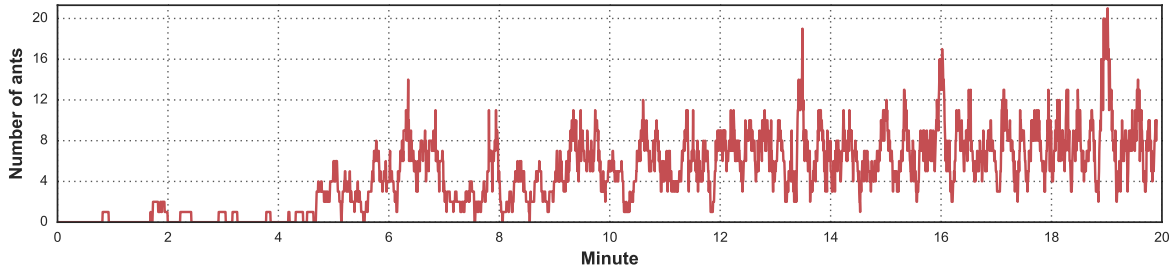
(a) Segmentation results of the ants on time frame 19:01. Color denotes each labeled object.
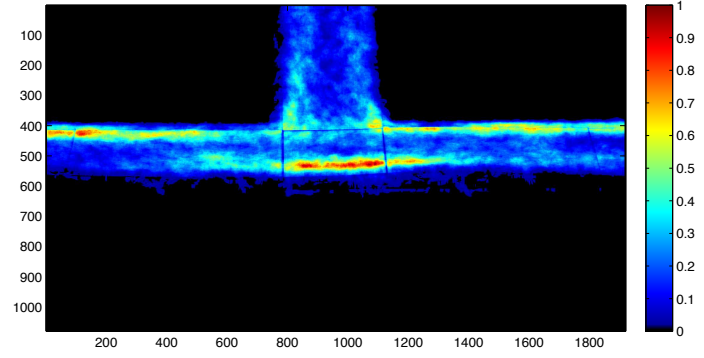
(b) Historical location of ants over the bridge shown as a cumulative sum of the individual ant positions. The colors denote the normalized sum at the last time frame.
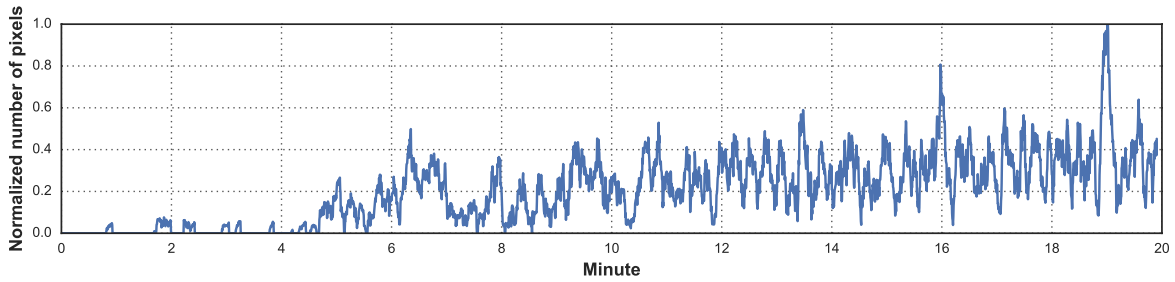
(c) Normalized number of foreground pixels by time.

(d) Number of ants present on scene by minute.

Fig. 4: Example of the extracted visual analysis.

For each frame i we computed the histogram of its reference area with a bin size equal to 100. Then we computed a pair of intensity reference bins $P_{i,1}$ and $P_{i,2}$ as the local maxima peaks from the first and second halves of the histogram. Starting from the second frame, we respectively defined the factor and sum adjustments as

$$f_i = \frac{P_{0,2}}{P_{0,1} - P_{i,1} + P_{i,2}} \qquad (3)$$

and

$$s_i = f \cdot (P_{0,1} - P_{i,1}) \qquad (4)$$

Subsequently, we equalized the frame i by thresholding the result of multiplying the pixels of frame i by the factor f_i and adding the value of s_i.

2) Segmentation: This was a three-step task that consisted in extracting foreground pixel blobs that might contained one or more ants. In the first step, the foreground pixels were extracted for each frame by subtracting the reference frame. Moreover, the regions outside the T-maze and the registered borders were removed by using a binary mask. During the second step, small segmented regions and remaining horizontal and vertical lines were removed by using simple morphological operations. Specifically, we used erosion and dilation for the small regions and closing for the lines. In the third step, the bounding box and centroid were computed for each blob.

3) Tracking: We implemented a nearest-neighbor tracking strategy, based on the distance between centroids. That is, we keep an ant track by assigning the blob with the closest
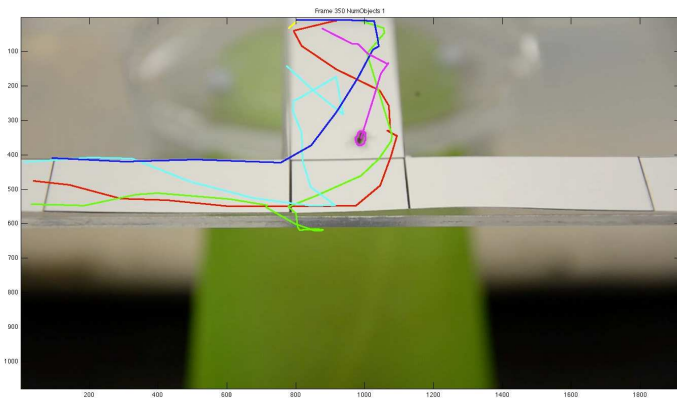
Fig. 5: One representative frame showing the tracking results. In this frame six tracks are visible.

distance between the centroids of two consecutive frames. In addition, a track was considered to start and end when an ant entered and exited the T-maze, correspondingly. Fig. 5 shows a sequence frame containing one tracked ant and five other left traces.

4) Measurement extraction and statistics: This task generated observation statistics based on the segmented blobs from all frames. One of the generated visualizations is the number of ants present per frame. Fig. 4a and 4d show an example of the labeled ants in the last frame and the plot of the number of seen ants by time, respectively. Another generated visual information is the normalized cumulative sum of the blobs of all images. Fig. 4b and 4c show the final result of the normalized cumulative sum and the plot of the normalized number of foreground pixels by time, correspondingly. The results were displayed as a video.

III. DISCUSSION

We presented an ant behavior analysis extracted from an experiment video sequence. The foraging experiment analyzed consisted of the observation of two food sources at opposite-ends of a T-junction bridge. As the food sources were depleted the ants could redirect their paths. The heatmaps of the tracks suggested that the ants favored the edges of the bridge as compared with the central regions. The temporal presence of ants on the bridge was calculated as the pixels in the foreground and the number of ants segmented per frame. This was assessed visually and corresponded to the ant activity. In the future, we expect to obtain a ground-truth to estimate the degree of segmentation and tracking.

Despite the fact that the video was not originally meant to be automatically analyzed, the results encourage further collaboration.

REFERENCES

[1] T. J. Czaczkes, C. Grüter, L. Ellis, E. Wood, and F. L. Ratnieks, "Ant foraging on complex trails: route learning and the role of trail pheromones in lasius niger," *Journal of Experimental Biology*, vol. 216, no. 2, pp. 188–197, 2013.

[2] C. Grüter, D. Maitre, A. Blakey, R. Cole, and F. L. Ratnieks, "Collective decision making in a heterogeneous environment: Lasius niger colonies preferentially forage at easy to learn locations," *Animal Behaviour*, vol. 104, pp. 189 – 195, 2015.

[3] C. Grüter, T. J. Czaczkes, and F. L. W. Ratnieks, "Decision making in ant foragers (lasius niger) facing conflicting private and social information," *Behavioral Ecology and Sociobiology*, vol. 65, no. 2, pp. 141–148, 2011.

[4] I. M. Grześ, "Ants and heavy metal pollution – a review," *European Journal of Soil Biology*, vol. 46, no. 6, pp. 350 – 355, 2010.

[5] J. Venkateswara Rao, P. Kavitha, N. M. Jakka, V. Sridhar, and P. K. Usman, "Toxicity of organophosphates on morphology and locomotor behavior in brine shrimp, artemia salina," *Archives of Environmental Contamination and Toxicology*, vol. 53, no. 2, pp. 227–232, 2007.

[6] J. Fabrega, S. N. Luoma, C. R. Tyler, T. S. Galloway, and J. R. Lead, "Silver nanoparticles: behaviour and effects in the aquatic environment," *Environment international*, vol. 37, no. 2, pp. 517–531, 2011.

[7] F. L. W. R. M. Beekman, "Long-range foraging by the honey-bee, apis mellifera l." *Functional Ecology*, vol. 14, no. 4, pp. 490–496, 2000.

[8] F. L. Ratnieks and N. L. Carreck, "Clarity on honey bee collapse?" *Science*, vol. 327, no. 5962, pp. 152–153, 2010.

[9] F. L. Ratnieks, "Trail pheromones: An integrative view of their role in social insect colony organization," *Annu. Rev. Entomol*, vol. 60, pp. 581–99, 2015.

[10] C. Grueter, R. Schuerch, T. J. Czaczkes, K. Taylor, T. Durance, S. M. Jones, and F. L. Ratnieks, "Negative feedback enables fast and flexible collective decision-making in ants," *PLoS One*, vol. 7, no. 9, p. e44501, 2012.

[11] T. J. Czaczkes, C. Grüter, and F. L. W. Ratnieks, "Negative feedback in ants: crowding results in less trail pheromone deposition," *Journal of The Royal Society Interface*, vol. 10, no. 81, 2013.

[12] J. A. Solis-Lemus, Y. Huang, D. Wlodkovic, and C. C. Reyes-Aldasoro, "Microfluidic environment and tracking analysis for the observation of artemia franciscana," in *26th British Machine Vision Conference*, 2015.

[13] K. M. Henry, L. Pase, C. F. Ramos-Lopez, G. J. Lieschke, S. A. Renshaw, and C. C. Reyes-Aldasoro, "Phagosight: an open-source matlab® package for the analysis of fluorescent neutrophil and macrophage migration in a zebrafish model," *PloS one*, vol. 8, no. 8, p. e72636, 2013.

[14] D. G. Lowe, "Object recognition from local scale-invariant features," in *Computer Vision, 1999. The Proceedings of the Seventh IEEE International Conference on*, vol. 2, 1999, pp. 1150–1157 vol.2.

[15] A. Vedaldi and B. Fulkerson, "VLFeat: An open and portable library of computer vision algorithms," http://www.vlfeat.org/, 2008.