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Mice and larvae tracking using a particle filter with an auto-adjustable observation model

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ABSTRACT

This paper proposes a novel way to combine different observation models in a particle filter framework. This, so called, auto-adjustable observation model, enhance the particle filter accuracy when the tracked objects overlap without infringing a great runtime penalty to the whole tracking system. The approach has been tested under two important real world situations related to animal behavior: mice and larvae tracking. The proposal was compared to some state-of-art approaches and the results show, under the datasets tested, that a good trade-off between accuracy and runtime can be achieved using an auto-adjustable observation model.

1. Introduction

Visual tracking of multiple objects is an essential task for many applications and has been successfully used in animal behavior analysis. Scientific experiments in laboratory with live animals need several hours or even days of constant observation by the researcher. The observation task for a long period of time by a human observer is a very boring and fatiguing work and most of the time the results obtained are not reliable or reproducible.

A variety of algorithms for tracking multiple objects has been proposed. Many of them are based on predictive filters, in order to achieve robustness to occlusion and real time performance. Predictive filters use a stochastic model of the tracked objects dynamics in order to propagate the state of the system, from frame to frame. The predicted state is combined with information derived from an observation model, to estimate the current state of the system (Funk, 2003).

This paper extends the work proposed in our previous work (Gonçalves et al., 2007a) with (1) an updated literature review, (2) a new set of experiments on another real-life problem (larvae tracking) and (3) an improved explanation and clarifications on the tracking approach proposed in our previous work.

The tracking approach proposed is based on particle filter augmented with an auto-adjustable observation model. This auto-adjustable observation model combines, dynamically, a connected component analysis and a k-means based model. In order to deal with situations where mice are in contact or under partial occlusions, the k-means algorithm (Har-Peled and Sadri, 2005; Malyszko and Wierzchon, 2007) is used. The k-means solve the problem of tracking with contact between objects, but with a relatively high processing time. Conversely, connected component analysis produces a faster tracker but can not handle situations where the objects are in contact. In order to obtain a balance between tracking precision and reduced runtime, in this paper it is proposed an observation model that can, automatically, change between k-means and connected component analysis. The dynamics model used in this paper is inspired in the random walk (Bartumeus et al., 2005) motion model, whose parameters have been set specifically for mice and larvae movements.

The tracker was analyzed using image shots in situations where the objects are both in contact or separated from each other. The particle filter performance was compared to that of human specialists, in the open-field experiment. This experiment proved to be an interesting way to compare tracking algorithms, as it provides ground truth data related to the objects spatial position over a observation section. Our proposal demonstrated to be correct

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when compared to ground truth data (collected by humans) and to be robust and efficient, when compared to other approaches (single observation model).

The paper is organized as follows. Section 2 describes the mice and larvae tracking problem. Section 3 presents related work on the application of computer vision to animal behavior identification and on particle filters. Section 4 briefly reviews particle filters and explains the auto-adjustable observation model. The experiments performed and the results of the proposed approach using the particle filter applied to mice and larvae tracking are described in the Section 5. Finally, the conclusions are presented and future works are discussed in Section 6.

2. Background

Computer vision has been increasingly used to automate scientific experiments in laboratory. Spink et al. (2001) describes how video-based tracking allows researchers to study the animal behavior in a reliable and consistent way for a long period of time to test new medicines on mice. The researcher is interested in how the behavior patterns change during exposure to pharmacological agents. In general, these activities are applied in large scale, using multiple doses and different animals, in order to ensure reliable statistics.

In particular, for measuring motion behavior (e.g. track length, velocity, acceleration), unusual movements that happen after long periods of inactivity and the ones that occur during many hours or days (daily behavior analysis, for example), video-based tracking is an interesting alternative to a human observer, who is unable to perform these tasks efficiently (Spink et al., 2001). In addition, automatic tracking does not suffer from fatigue or distraction and this approach eliminates the subjectivity when various observers classify the same action performed by the animal. This paper presents two different real world situations of animal behavior analysis: the mice and larvae tracking.

Tracking multiple mice is an interesting task because mice are deformable and, in some cases, indistinguishable objects. The animal behavior along these experiments may be recorded, automatically or semi-automatically, in video. During the experiment, the researcher observes the animal and records information about actions related to specific behavior of interest. Video-based tracking can aggregate automatic pattern recognition, applied to the captured images, to extract measurements from animal behavior.

Tracking multiple larvae has recently turned into a very important task for public health. In Brazil, Dengue causalities, according to the Ministry of Health, affected around 128.13 cases by 1,00,000 inhabitants. Dengue control has been limited to combating the vector using synthetic and biological insecticides. However, due to continuous use, the vector, *Aedes aegypti*, has become resistant to some chemical products. The ecological damage produced by synthetic insecticides has motivated scientific research towards finding active products of botanical origin, and a number of species have been investigated.

In order to access the efficacy of this products, bioassays are carried out as five replicates in a climate-controlled environment. Twenty third-instar larvae of *A. aegypti* are placed in a 25 ml test solution. Essays are conducted using the same number of larvae in a DMSO-distilled water solution. The larvae mortality after 24 h are recorded. Total absence of larval movement as well as dark body color and cephalic capsule are used as an indicative of death. A computer vision system is being devised to automate this process and will provide greater reliability, reproducibility and access to information not easily obtained by humans, as the precise time of death of each larva. As the larva is placed in a liquid solution, that is constantly moving due to the live larvae, the "absence of movement" can not be easily identified using simple standard computer vision techniques.

In both cases, mice and larvae, tracking multiple objects is required. Basically, tracking multiple objects consists in determining which and how many objects in the scene will be tracked and then locating each one of them in consecutive frames. This task receives a special attention in computer vision; however, it is still an open and challenging problem due to the variation in the conditions of lighting, presence of noise and potentially ambiguous conditions, such as occlusion of multiple similar objects. Some examples of applications of tracking multiple objects are tracking multiple animals to automate experiments with laboratory animals (Branson and Belongie, 2005), social insect interaction analysis (Zia Khan Balch and Dellaert, 2003; Morais et al., 2005), monitoring people for tracking players (Okuma et al., 2004), identification of 3-d human motion (Choo and Fleet, 2001).

3. Related work

There are many computer vision works approaching the problem of automatic animal behavior analysis and this section briefly report on some of these works. This section also presents some recent work related to the use of particle filters in tracking multiple objects in images.

3.1. Automatic animal identification and behavior analysis

Automation of animals identification and behavior analysis, both in controlled or in wildlife situations, is becoming a very important topic in Computer Vision. For instance, Burghardt and Campbell (2007) combined several computer vision techniques, like feature prediction trees and shape contexts in order to find and identify African penguins living in a colony. Using a new class of human psychology inspired structural descriptors, Chia et al. (2008) presented some promising results on the automatic identification of four-legged animals, including cows and horses. The identification of snakes attack behavior has been tackled, using a Hidden Markov Model framework, by Gonçalves et al. (2007b).

Haar-like features and AdaBoost classifiers, integrated with a low-level feature tracker, were used in (Burghardt and Calic, 2006) to detect and track lions on wildlife videos. Two recent works tackle the problem of insects identification and tracking using techniques based on invariant moments and concatenated histograms of local appearance features (Kumar et al., 2007; Larios et al., 2007). Other species whose automated monitoring are being pursued using computer vision techniques include fishes (Zhou and Clark, 2006; Morais et al., 2005), bears (Wawerla et al., 2009) and birds (Figueiredo et al., 2003; Tweed and Calway, 2002).

3.2. Particle filters

Particle filter has been extensively used in tracking multiple objects (Hue et al., 2001; Moreno-Noguer and Sanfeliu, 2005), employing visual (e.g. color, texture), geometric (e.g. contours, shape) and motion features (Moreno-Noguer and Sanfeliu, 2005; Okuma et al., 2004; Hue et al., 2001). In (Moreno-Noguer and Sanfeliu, 2005) a robust framework for tracking rigid and non-rigid objects was developed. The particle filter implementation was based on visual and geometric features. The framework was evaluated in two experiments, a book boundary tracking and a moving leave, in situations that other algorithms may fail.

Particle filter has been used to track objects in different domains. Chakravarty and Jarvis (2006) apply particle filter to track multiple persons using visual features and Vacek et al. (2007) use particle filter and lane detection to track road marking in an autonomous vehicle. Krahnstoever et al. (2001) use particle filter for tracking articulated objects.

Branson and Belongie (2005) propose an algorithm for tracking the contours of multiple mice. The experiments used frames captured from a side view of the cage where the mice are inserted and frequently occlude each other. Their algorithm that combines multiple blob detection and a contour tracker demonstrates an acceptable performance, mainly in occlusion. In (Morais et al., 2005), a particle filter tracker is applied to track fishes. Tracking is based on the multitarget likelihood function and a set of 2000 particles.

Many new extensions to the particle filter approach, that can handle the very large search spaces associated with articulated objects tracking, are being developed. Some of this extensions are the Subspace Hierarchical Particle Filter (Brandão et al., 2006) and the Smart Particle Filters, which wraps a particle filter around multiple Stochastic Meta-Descendent trackers (Bray et al., 2007). Other extensions, that share the same motivations as the proposal presented in this paper, are the ones that dynamically combine different observations or dynamics models into the same framework, as the work of Gao et al. (2008) and Zou et al. (2008). The use of k-means and connected component analysis as observation models has been proposed in (Micilotta, 2004), however, differently from the work presented here, in their proposal there was no provision on how one of these models should be chosen. In our work, the system automatically decide, heuristically, when k-means or connected component analysis are used. In this way, during tracking, the system can change many times between k-means and connected component analysis, providing a balance between tracking accuracy and runtime speed.

4. Particle filter with an auto-adjustable observation model

The following sections describe the particle filter observation and dynamic models used in this work.

4.1. Auto-adjustable observation model

The observation model combines a k-means based and a connected component analysis based model. The connected component analysis based model uses the blobs of the segmented image to infer the center of mass and the other parameters of an ellipse that approximates the mice contour. This inference is easily carried out using an observation model based on connected component analysis when the objects have no contact among themselves. The main problem for extracting the system state, in this case, is the constant contact between objects, as it is shown in Fig. 1, in the mice domain problem. The contact between objects results in a single blob in the image that makes the task of differentiating among the several objects very difficulty.

To deal with this special situation of merged objects owe can use some a priori information about the object model in order to reduce the observation uncertainty. In this situation, the observation model is dynamically modified to infer the parameters of k objects in the scene using the standard k-means algorithm (Har-Peled and Sadri, 2005; Malyszko and Wierzchon, 2007). The main idea is to apply k-means to cluster the pixels in the merged blob in order to split the objects, considering that k, the number of objects in the scene, is known. This is usually the case in problems related to laboratory animals tracking.

To reduce the processing cost, the center of each object is initialized with its position in the previous frame. Each pixel marked as foreground, in the segmented image, is attributed to the closest center using the Euclidean distance from that pixel position to each center. Fig. 1 shows an example of the results obtained using this technique.

After the attribution step, the center of each pixel group is recalculated using the mean of the pixels positions. The attribution and the calculation step are repeated until it does not have a significant change, τ, between the current center and the previous one. In this application, five iterations are necessary, on average, and 0.1 was experimentally used for τ. The parameters of the ellipse are extracted using the pixels attributed to the center of each group.

The problem with k-means, in comparison to the standard connected component analysis algorithm, is related to its higher processing time. The observation model is then changed, dynamically, between k-means and connected component analysis, based on a threshold that heuristically identify animals contact situations, so that k-means is used only when needed, resulting in an auto-adjustable observation model. The experiments in the next section will show that a good trade-off of tracking precision and computing time consumption can be attained with this approach.

4.2. Dynamic model

To obtain a robust particle filter, it is important to model the dynamic behavior of the objects in the system. Both the mice and larvae movement, tackled in this paper, can be fit by random walk models that are probabilistic discrete step models that involve strong simplifications of an animal movement. It consists in a discrete series of displacement events separated by reorientation events (Bartumeus et al., 2005).

The dynamic model inspired by random walk motion characterizes a random motion and can be described as:

$$f(\vec{x}_k) = \vec{x}_{k-1} + \vec{r}(\sigma) * \vec{V} \quad (1)$$

where $\vec{r}(\sigma)$ is randomly generated through a Gaussian number generator with standard deviation σ and V is the velocity of the object.

Fig. 1. Original image (left), segmented image using background subtraction (center) and the results of the k-means clustering procedure with results shown in ellipses (right).

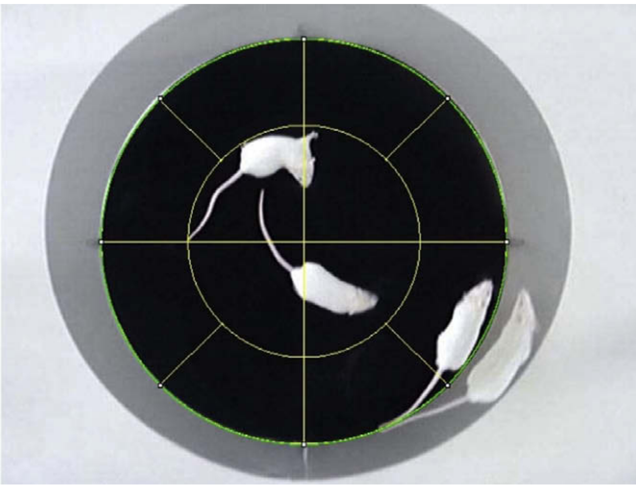

Fig. 2. The 12 equal area regions used to provide a coarse tracking annotation for the open-field mice experiment. There is only three mice in this arena, the fourth mouse in the lower right corner is just a mirror reflection due to the acrylic wall that surrounds the arena. This mirror image problem is solved using a simple segmentation procedure that isolate the circular arena.

This model is interesting for objects that do not possess a standard movement. However, the parameters chosen should represent the dynamic of the object, as the velocity. These parameters should be carefully chosen once a high or low velocity can diminish the performance of the particle filter.

5. Experiments and results

Two sets of experiments have been devised to access the performance of the proposed approach. The first, using mice videos, has been first presented in (Gonçalves et al., 2007a), and has been reproduced here, with some presentation improvements. The second is a new experiment that used images of *Aedes aegypt* larvae obtained during real essays aimed at testing the efficacy of a new biological insecticide. In both experiments, the state of the system, in the particle filter, is represented by a multidimensional variable $\vec{x}_k = [x\,y\,m_e\,m_i\,\theta]$, that approximates the contour of each animal by an ellipse, where (x, y) is its center point, m_e and m_i are the major and minor axis, respectively, and θ is the inclination angle.

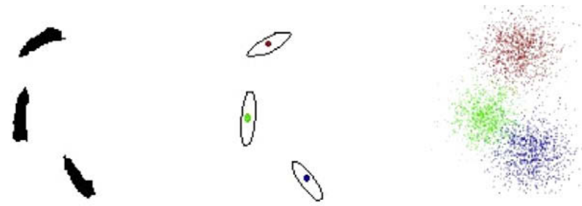

Fig. 4. Example of mice not in contact. The particles distribution for each of the three particle filters associated with each mouse is shown in different colors (right). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

5.1. Mice tracking

In the first experiment, the problem to be investigated is the tracking of four mice in an experiment called open field (Eilam, 2003). This experiment is carried out in a cylindrical arena surrounded by acrylic walls with approximately 30 cm of height. The arena of the experiment is divided in 12 regions with the same area. The top view of the experiment with the 12 regions is shown in Fig. 2. Each mouse has been associated with a different particle filter, all sharing the same observation and dynamic model.

In order to evaluate the efficiency of the implemented particle filter, the tracking results where compared to that of a trained human, using a standard methodology developed to track, without automation and using a coarse position system, the mice in the arena. This methodology associates one of each of the 12 arena regions to each mouse, in accordance to a visual approximation of its center of mass. The region supplied by the tracking algorithm is compared to the ground truth region (assigned by humans), providing a correct classification rate. A special situation occurs in images where the manual marking is not easily found, i.e., situations where the mouse is in a border between regions and the specialist is not sure about which region the mouse is in. In this situations, the ground-truth region was chosen to be the region recorded in the previous frame, following the recommendation of an expert from the UCDB's Biotechnology Department (Schiaveto, 2007).

In this experiment, image shots, taken from a camera placed over the open field have been used. Shots where the mice are in contact and in partial occlusion have been chosen. This image shots have been extracted using videos recorded by a digital camera

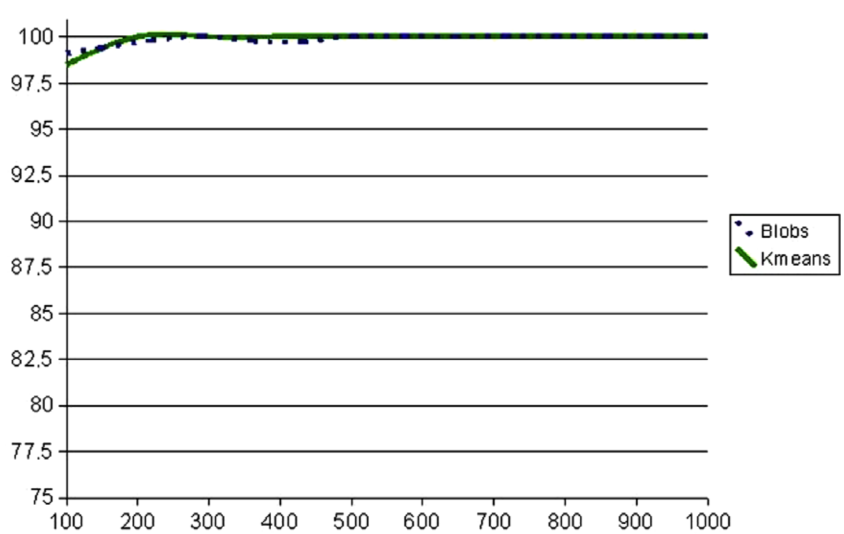

Fig. 3. Number of particles versus correct classification for mice without contact.

Canon Powershot A80, with resolution of 320 x 240 pixels. Later, the frames have been segmented using background subtraction. All the tests have been performed using a computer with a P4 2.8 GHz processor, 512MB of RAM and a Linux operating system with Kernel version 2.4.

Four quantitative and one qualitative particle filter parameters variations were explored during the experiments. For the dynamic model, the parameters varied were the standard deviation σ and the velocity in the X and Y direction of the random walk model. For the observation model, one based on connected component

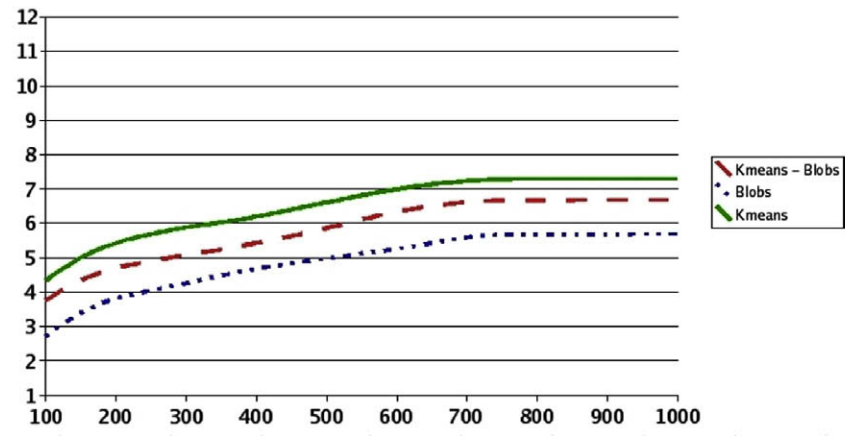

Fig. 5. Runtime (in seconds) over the number of particles (horizontal axis). The results using the auto-adjustable observation model is shown in red and is labelled "*k* means-Blobs". (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

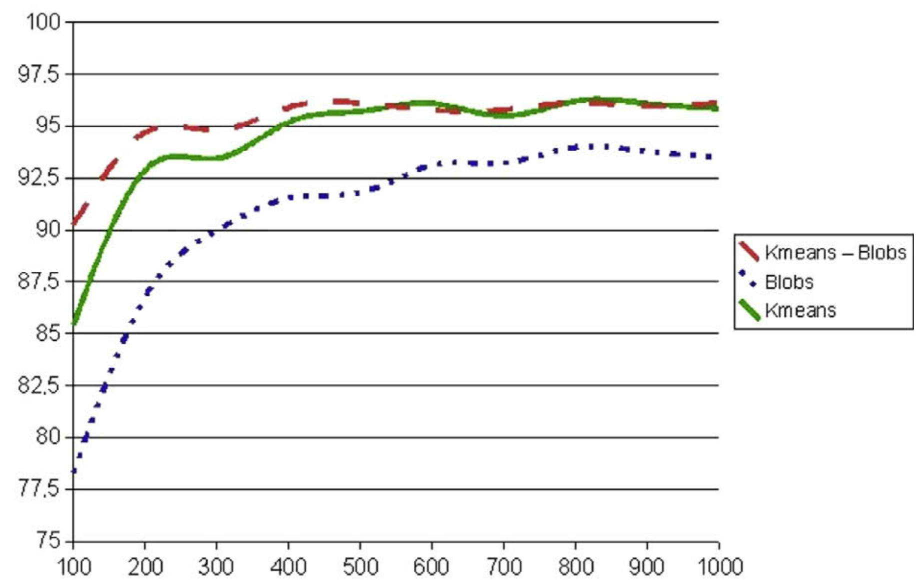

Fig. 6. Number of particle versus correct classification rate for mice in contact.

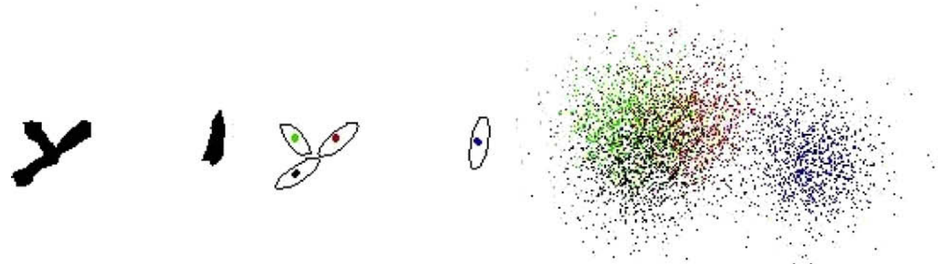

Fig. 7. Example of tracking results when mice are in contact.

analysis, another based on the k-means algorithm and a third one based on the proposed auto-adjustable observation model, combining the two previous models, were tested. The last parameter tested were the number of particles of the particle filter. The particle filter was evaluated on 54 images where 4 mice move separately, that is with no contact and 96 images where mice move in clusters, in contact with others.

The results are presented in Figs. 3, 5 and 6. In Fig. 3, the graphic presents the variation in correct classification rate as the number of particles grows for image shots where the mice are not in contact. Only the mean correct classification rate for the other three quantitative parameters variations is plotted. Both models, connected components and k-means, presented classification rates above 97%, with just 100 particles. The graphic shows that the observation models based on connected components analysis and k-means achieves practically the same good performance when the mice move with no contact with others (see an example in Fig. 4).

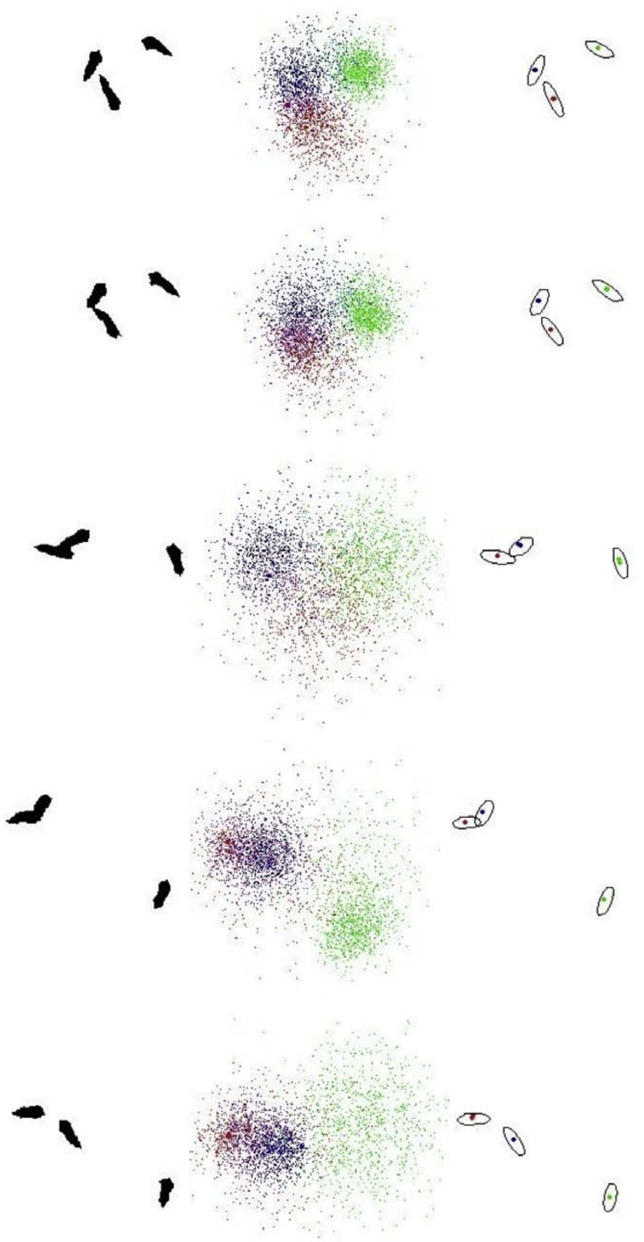

Fig. 8. Example of tracking results for three mice and using 1000 particles in the filter.

The relation between the number of particles and the runtime (in seconds) is presented in Fig. 5. The observation model based on connected component analysis was faster in relation to the model based on k-means, but both presented a constant runtime after 700 particles. The higher runtime of the k-means model in Fig. 5 and the similarity in correct classification rate results for both models in situations where the mice move separately (as in Fig. 3, for example), inspired the present approach that combines the two models. Experiments using this combination (the auto-adjustable observation model) were carried out in the following way: when the image does not have mice interaction the observation based on connected components is utilized and when the image contains mice in contact the observation based on k-means is used. The system recognizes mice interaction when the quantity of blobs in the image is less than the previously known quantity of mice to be tracked. That combination reduces the running time, shown in the Fig. 5, and maintain a good correct classification rate (Fig. 6).

In Fig. 6, the graphic presents the result for image shots with mice moving as a cluster. Tracking a cluster of mice, as illustrated in the Fig. 7, is a difficult task, also for humans. For contact situations, the observation model based on connected component analysis does not achieve a good performance. However, the model based on k-means and in the combination between the models solve this problem, achieving better results. The model based on k-means with 400 particles achieved a mean of 95% correct classification rate. In contrast to the first model, the model based on the combination between k-means and connected components analysis achieved a mean of 96% correct classification rate. Although both techniques present similar correct classification rate, the combination of k-means and connected components analysis outperformed the k-means, once it obtained a faster runtime (see Fig. 5).

Fig. 8 shows the tracking results for three mice in a sequence of images where mice start moving separately, then they cluster together and afterwards they split again. For each situation the

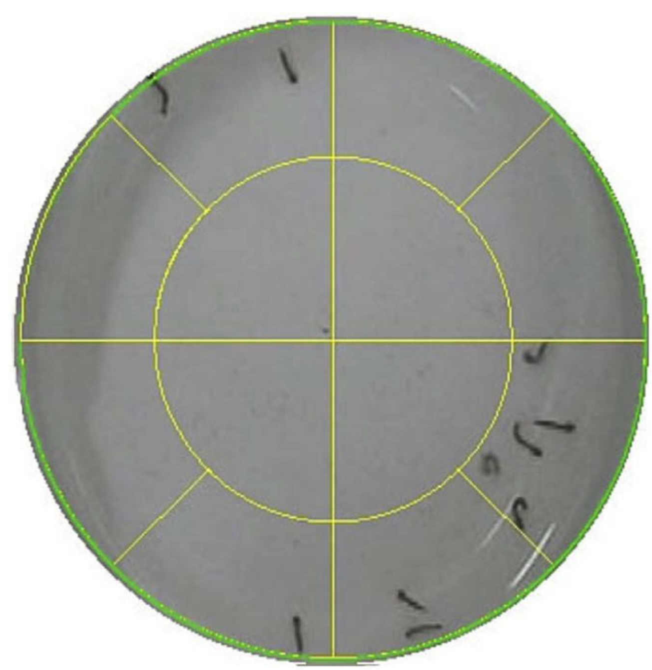

Fig. 9. Regions used in the experiments with larvae.

particles distribution and the ellipses representing the mice are presented. It is worth noting that even when the mice are all clustered together, the combination of particle filter and *k*-means, as proposed in this article, obtained good tracking results.

5.2. Larvae tracking

The problem tackled in the second experiment is to track ten larvae placed in a 25 ml liquid solution. The top view of the experiment with the 12 regions, as in the experiments with mice, is shown in Fig. 9. Each larva has been associated with a different particle filter, all sharing the same observation and dynamic model. The same ground-truth comparison methodology used in the mice experiment were used in this second experiment.

As in the first experiment, image shots were taken from a camera placed over the animals container. Image shots where the

Fig. 12. Example of larvae not in contact.

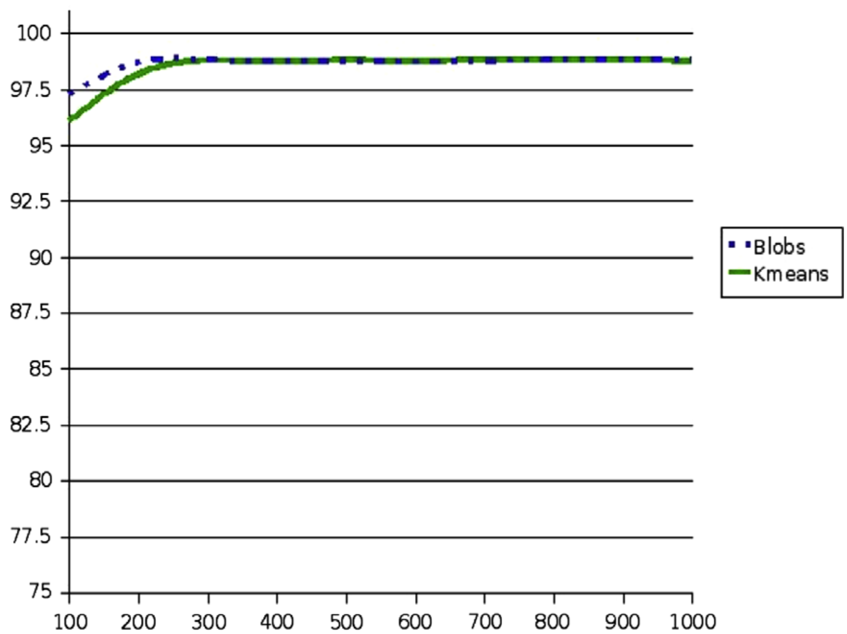

Fig. 10. Number of particles number versus correct classification rate for larvae not in contact.

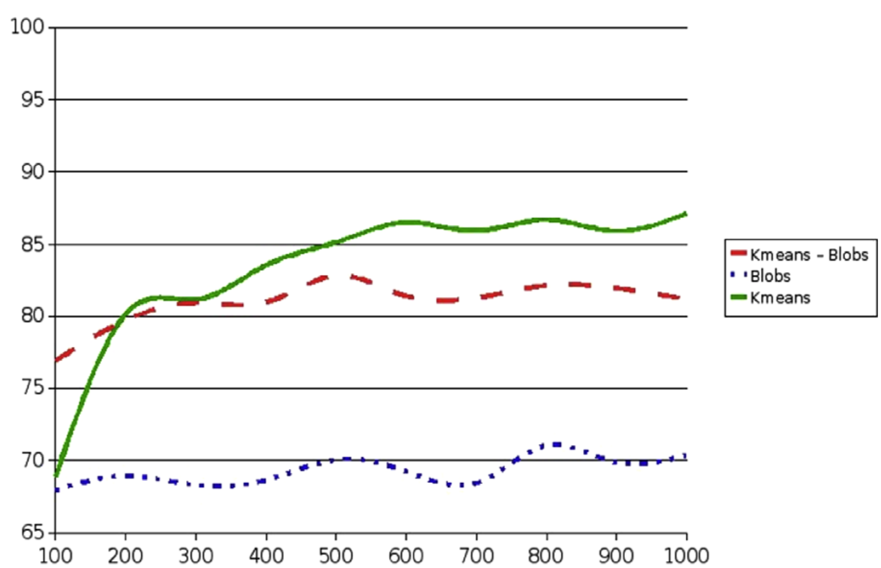

Fig. 11. Number of particles versus correct classification for larvae in contact.

larvae are in contact and in partial occlusion have been selected. These image shots, with a 720×480 pixels resolution, have been captured using a webcam Logitech Quickcam Pro 4000. Later, the frames have been segmented using background subtraction. All

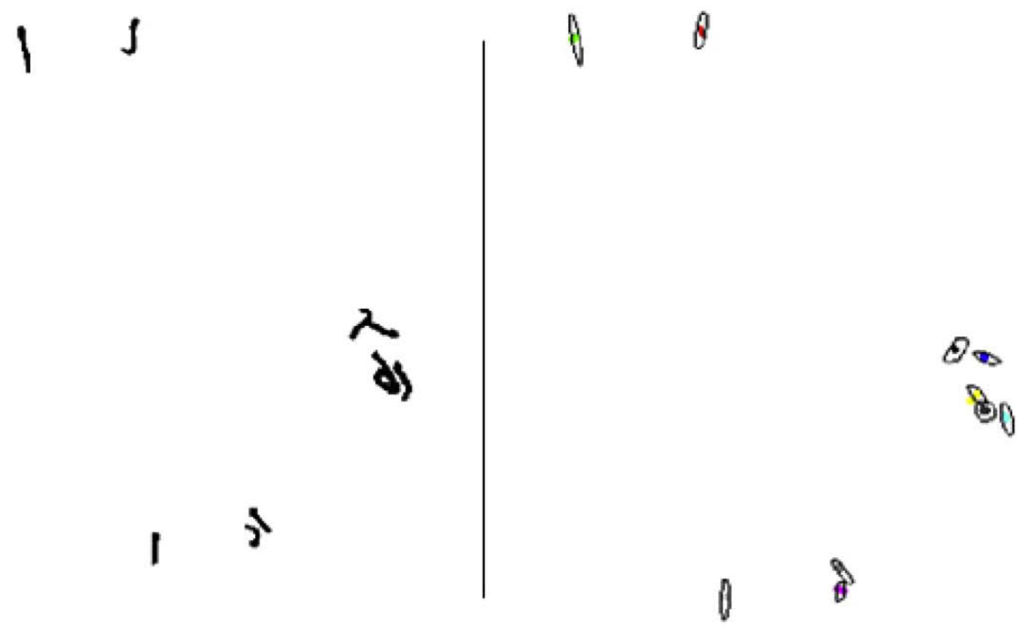

Fig. 13. Example of larvae in contact.

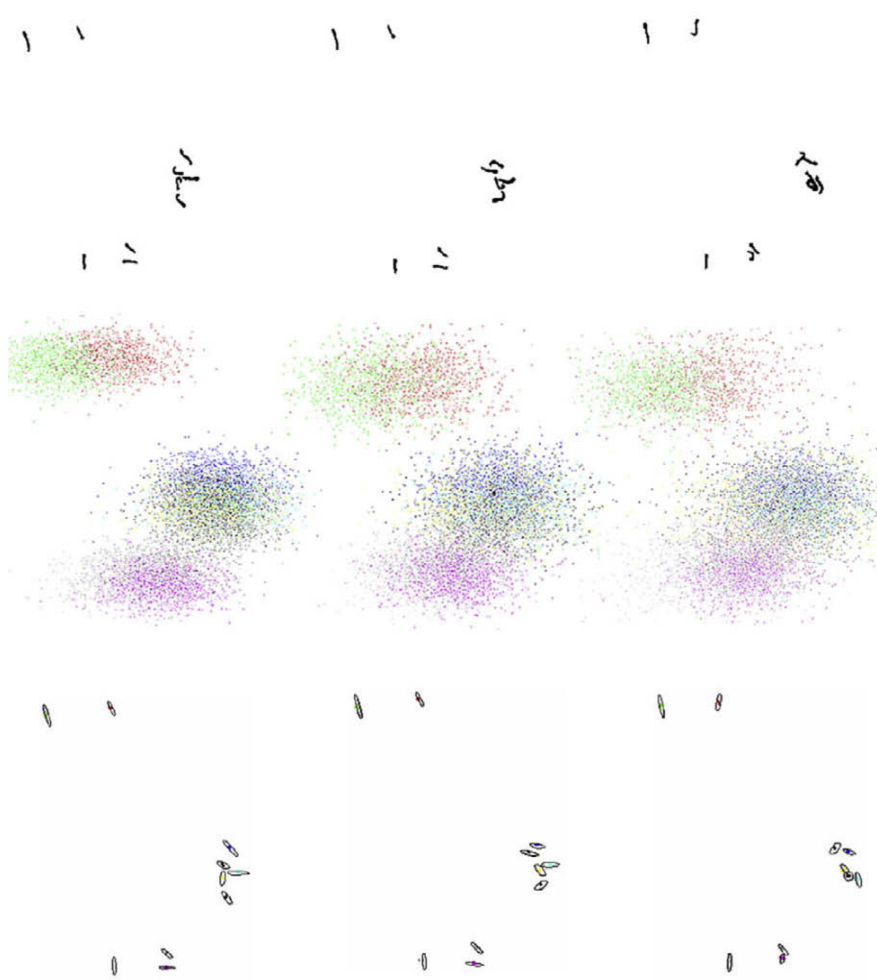

Fig. 14. Example of tracking results for 10 larvae and 1000 particles for each particle filter.

the tests have been performed using a computer with a dual-core processor, 1GB MB of memory RAM and an Linux operating system with Kernel version 2.6.

The same parameters as in the first experiments were varied. The filter was evaluated in 50 images in which some of the larvae move as a cluster, in contact with others, and in more 50 images in which all the larvae move separately, that is, with no contact or occlusion.

The results are presented in Figs. 10 and 11. In Fig. 10, the correct classification rate over the number of particles, when larvae are never in contact, is shown. As in the first experiment, the correct classification rate corresponds to the mean values over the other free parameters. Both nonauto-adjustable observation models present classification rates above 95%, with just 100 particles. Similar performance is achieved using the auto-adjustable model. Fig. 12 shows an image frame when larvae are not in contact.

In Fig. 11, the graphic presents the correct classification results for images where larvae are in contact. In this situation, the graphic shows that an observation model based on simple connected component analysis does not achieve a good performance. However, both the *k*-means and the auto-adjustable observation model provide better correct classification rates. The model based on *k*-means, using 500 particles, achieves a mean of 85% correct classification rate, whereas the auto-adjustable model reaches a 83% mean correct classification rate, a much better rate than the 70% achieved using connected component analysis (See Fig. 13).

Fig. 14 shows an example of the tracking results for ten larvae in a sequence of 3 image frames where some larvae start moving separately and then they cluster together. For each situation the particles distribution and the ellipses representing the larvae contours are presented. It is worth noting that, as with the mice example, even when the larvae are all clustered together, the combination of particle filter and *k*-means, as proposed in this article, obtained good tracking results.

6. Conclusion and future work

This paper showed an implementation of the proposed auto-adjustable observation model, that combines different observations models in a particle filter framework, applied to the tracking of multiple mice and larvae. Experiments have shown that an observation model based on the combination between *k*-means and connected component analysis, dynamically chosen, can lead to higher correct classification rates than a model based only on connected components analysis and almost as high as the one based on *k*-means, without a great penalty in processing time. The use of *k*-means during the observation phase of the filter showed to be important for partial occlusion robustness. However, when mice move separately, the results are basically equal. The use of standard *k*-means has the drawback that it cannot separate clusters that are non-linearly separable in input space. However, the typical mice clustering situations, as illustrated in Figs. 1 and 8, are linearly separable. The problem of optimal locality, associated with the standard *k*-means formulation can be handled using good initial values. In the experiments discussed in this paper, the use of previous estimated positions of the object and the auto-adjustable observation Model, has proven to be a good choice.

The main contributions of this article are the use of a dynamical combination of the *k*-means and the connected component analysis in the observation model of a particle filter and the empirical evaluation of this approach using two important real-world problems. Another contribution is the tracking evaluation procedure, based on a standard test much used in pharmacological research. For future research, it would be interesting to include information related to the contour of the objects in the state model, and to use variable velocity in the dynamic model, calculated using optical flow techniques, for instance. It would also be interesting to expand the tests using a larger amount of images, with different kinds of animals and environments. The use of a more sophisticated cluster technique, in place of *k*-means, should also be investigated.

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