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An algorithm to track laboratory zebrafish shoals

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ABSTRACT

In this paper, a semi-automatic multi-object tracking method to track a group of unmarked zebrafish is proposed. This method can handle partial occlusion cases, maintaining the correct identity of each individual. For every object, we extracted a set of geometric features to be used in the two main stages of the algorithm. The first stage selected the best candidate, based both on the blobs identified in the image and the estimate generated by a Kalman Filter instance. In the second stage, if the same candidate-blob is selected by two or more instances, a blob-partitioning algorithm takes place in order to split this blob and reestablish the instances' identities. If the algorithm cannot determine the identity of a blob, a manual intervention is required.

This procedure was compared against a manual labeled ground truth on four video sequences with different numbers of fish and spatial resolution. The performance of the proposed method is then compared against two well-known zebrafish tracking methods found in the literature: one that treats occlusion scenarios and one that only track fish that are not in occlusion. Based on the data set used, the proposed method outperforms the first method in correctly separating fish in occlusion, increasing its efficiency by at least 8.15% of the cases. As for the second, the proposed method's overall performance outperformed the second in some of the tested videos, especially those with lower image quality, because the second method requires high-spatial resolution images, which is not a requirement for the proposed method. Yet, the proposed method was able to separate fish involved in occlusion and correctly assign its identity in up to 87.85% of the cases, without accounting for user intervention.

1. Introduction

The zebrafish have been widely adopted both as a genetic and a biological model organism in neurobehavioral research due to its great scientific value, such as the study of gene function [1,2] and human diseases [3–5]. One of the key aspects of this species is its robust social behavior characteristics, which plays an important role in understanding several human brain illnesses like schizophrenia [6] and autism [7].

A common approach to studying the social behavior of zebrafish in the laboratory is through the capture and analysis of motion data of each individual in a shoal [8]. As a consequence of the recent advances in hardware technology observed in past years, the use of video tracking applications for both data acquisition and analysis have become quite popular [9–11].

When it comes to object tracking, what we are looking for is a means to retrieve the object's position in every time slice, so we can successfully reconstruct its trajectory by the end of the analysis. Tracking individual fish in a controlled environment is quiet simple, and there are several methods and tools found in literature that can solve this problem [23,24]. On the other hand, tracking multiple targets has proven to be a far more challenging task, due to the frequent occlusions between fish, i.e., when two or more individuals cross or touch each other [25].

When applying the tracking task to multiple objects, there are mainly two important problems that should be addressed: (1) the **object detection**, which deals with identifying the presence of objects of interest within an image; and (2) the **object identification**, which deals with assigning the correct identity to each object. Since shoaling behavior experiments are evaluated in a controlled environment, the images obtained from the fish tank usually have a high contrast between the fish and background. So, object detection can be easily achieved by several known methods [30–33]. In contrast, there is no simple method to distinguish objects that share a lot of visual features, such as the zebrafish, especially when the image sequence obtained from the fish tank has low spatial resolution.

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The main challenge faced by current multi-object tracking methods is to provide a robust method for the identification problem, especially after overlapping episodes. Kato et al. [14] applied mathematical morphology operators over blobs containing more than one fish, in order to separate them. Successive erosion operations are applied until the main blob is split into n objects, where n is the number of objects composing the main blob. This technique works well to handle most partial occlusion problems; however, it is not suited for more complex scenarios, such as full occlusions. On the approach presented by Escudero et al. [15], the occlusions are ignored. A fingerprint is extracted for every single fish in a small portion of the image sequence and in the following images they are matched with each fish candidate. The algorithm selects the individuals identity based on the best match with a particular fingerprint and creates a *tracklet* when the candidate does not match with any fingerprint. A tracklet is a portion of the full trajectory performed by an individual. So, in order to build the complete trajectory, several tracklets belonging to the same fish are connected at the end of the process. The reliability of this system depends on how well the environment is prepared, requiring a laborious setup. Besides, the produced fingerprint may not work properly to distinguish fish with similar shapes, colors and textures, as is the case of zebrafish.

Several authors aim to model the fish behavior by means of different equations, in order to generate hypotheses about its future position [16–18]. Delcourt et al. [16], for example, applies a set of simple linear equations based on the previous frame, nonetheless, this method does not consider the object's dynamic behavior and fails when one or more fish slow down or remain too close for a few frames. An alternative approach is to use methods such as the Kalman Filter [17] or the Particle Filter [18], which also consider the possible errors associated to the estimates produced for each fish. In these methods, a higher weight is given to estimates with higher certainty.

Since occlusion handling is the most common and challenging problem faced by techniques based on a single view of the scenario, some authors proposed techniques based on multiple cameras or multiple views of the scenario. In the work of Zhu and Weng [19], the additional views are obtained by a set of mirrors positioned around the fish tank. Two problems arise from this approach. First, a laborious setup is necessary to place the mirrors in their respective positions. Second, the processed images hold different views of the same scenario, which consequently reduces their individual spatial resolution. Still based on a single camera, Laurel et al. [13] uses two light sources to cast the shadows of a single fish at the bottom of the tank. The spatial 3D position of the fish is then extracted, based on its 2D position, on the casted shadows and on the information from the light sources. The problem of this technique is that when adding more fish, the occlusion can occur not only between fish, but also the generated shadows. In the work of Hai Shan Wu et al. [21], the authors take advantage of multiple physical cameras to track a group of fruit flies. Although the use of multiple cameras helps to solve some occlusion scenarios, as is the case when two fish overlap in one of the views but not in the others, it requires a laborious procedure to retrieve the cameras' relative position [27], which may be a hindrance for the widespread adoption of this approach.

The main contribution of the present work is a multi-object tracking algorithm for zebrafish tracking that addresses most of the above-mentioned challenges, such as occlusion handling, images with lower spatial resolution and simple set up configuration. In the proposed method, the fish are represented as blobs and, similarly to Quian et al. [17], there is a Kalman Filter instance responsible for keeping track of each animal along the experiment. In occlusion cases, when there are blobs containing more than one fish, there is an attempt to rebuild the poses of individuals contained in the blob. This reconstruction is achieved by using morphological dilate operations with dynamically-generated structuring elements for each individual, according to its movement direction and mean area in the previous frames. The algorithm was evaluated on four video streams with different numbers of fish and different spatial resolutions, and the results show an overall success rate in assigning the correct identity to individuals – ranging from 99.25% to 99.81% and a success rate of 85.1% in correctly separating individuals in occlusion.

Furthermore, as can be seen in Section 4, the proposed algorithm was compared against two other shoaling fish trackers [15,16] and it has been shown to outperform both trackers. These results indicate that the proposed algorithm is capable of keeping the correct reference of the blobs before, during, and after occlusion events, even when provided with lower spatial resolution images. Moreover, it is easy to set up and no special configuration is required. The overall process is illustrated in Fig. 1.

This article is organized as follows: A brief discussion about the Kalman Filter applied to fish tracking is presented in Section 2, while the detailed description of the proposed algorithm is found in Section 3. Sub-section 3.1 describes how the objects are detected and lists the set of features extracted for each candidate-blob. The best candidate-blob for each tracker instance and the blob partitioning methods are detailed in Sub-sections 3.2 and 3.3, respectively. In Sub-section 3.4, the step responsible for updating the instances' history is presented. The evaluation of the proposed method and a discussion about the results obtained are described in Section 4. Finally, concluding remarks are outlined in Section 5.

2. Kalman Filter for fish tracking

The Kalman Filter can be seen as a collection of equations that are used to estimate how a set of variables of interest, named as *state*, evolves over time. It uses those variables' history and a series of noisy observations to generate estimates about their current state [22].

In order to generate a valid estimate, the filter goes through a two-step process: **prediction** and **correction**. At the prediction step, the filter generates an initial estimate $\boldsymbol{X}_t^-$ based on the object's previous state. At the correction step, an observed measurement z_t is obtained and it is used to correct the initial estimate, resulting in the final variable's state

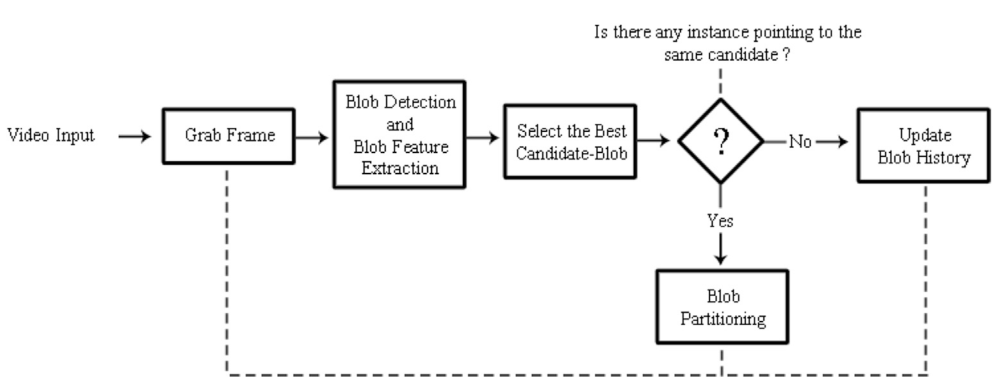

Fig. 1. Overview of the proposed algorithm.