SKELETON ANALYSIS OF POPULATION IMAGES FOR DETECTION OF ISOLATED AND OVERLAPPED NEMATODE C.ELEGANS

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ABSTRACT

In biotechnology, using image processing techniques to extract suitable information from C.Elegans nematode worms has recently drawn an increasing research interest. Many important problems in this area have not been solved yet, especially detection of each individual in population images. In this paper, we propose a method to detect individuals in population images. Our method is based on three steps: Pre-processing where a smooth skeleton is obtained from the image, Splitting skeleton into branches and angle calculation of each branch and finally merging the branches in order to detect each individual in the image. The outcome of the proposed method is a vision algorithm with 7.9% False Rejection Rate (FRR) and 8.4% False Acceptance Rate (FAR) on a database of 255 isolated and overlapped worms.

1. INTRODUCTION

In biotechnology, visualization of large number of micro-organisms is a necessary step for studying the effect of chemical compounds on complex eco-systems, finding growth rate and movement patterns, describing the culture composition and recently using fluorescent markers measuring the distribution of proteins and organ development. Nematoda phylum is one of the most important families of micro-organisms which abundantly exists in terrestrial and marine environment and also as a guest inside large number of plants and animals. A famous member of this family named Caenorhabditis Elegans (C.Elegans) used as a model organism [1] has attracted increasing research and commercial interests in ecology, molecular and developmental biology. C.Elegans is an unsegmented bilateral symmetric worm with wide thickness in the center and narrow near the head and tail. Because of its simple and featureless shape, detection and characterization is a challenging task for computer vision algorithms especially in presence of large number of specimens with strong overlapping between each other and with the plate.

Although image processing tools are utilized widely in daily lab work, the major part of the process is still labor intensive. Commercial software often employs semi-automatic approaches in order to effectively reduce the amount of time spent on analyzing single images with a few number of specimens. For video-sequences and massive population images these semi-automatic approaches are not suitable and further automation is necessary in order to process the higher amount of image data efficiency. As mentioned in [1], the need of high-throughput visualization of worms to fully describe biological processes on a quantitative level is still very much in demand. Recently, several integrated machine vi-sion systems have been developed. The goal of such systems is tracking a significant number of individual organisms and measuring parameters such as size, mobility, shape features, or fluorescent marker distribution as a function of time. [2] introduces a single worm detection algorithm which uses contour curvature patterns of the image skeleton to find the worm's head and tail. Morphological aspects of internal organs and an interaction detection procedure are used for nematode classification in [3]. The first work on C.Elegans behavioral phenotypes classification was done in [4] for motion pattern identification by means of a single nematode tracking system, morphological operators with grey scale thresholding and geometrical related features. Nematode population analysis based on basic image processing algorithms was introduced in [5] in a semi-automatic manner. Movement of a single worm is recorded in [6] by means of a motor-based tracking system, which makes the worm in the center of the video frame. The head and tail of the worm are extracted and 94 geometrical features are calculated for every frame. A Random Forests classifier identifies up to 15 different mutant types using these features. The accuracy of the method on a database of around 1600 individual nematode video sequences is about 90% . In [7], detection of worms in population images has been addressed using energies of optimized active contours to discover common worm patterns in the image. The extracted patterns are entered to a probabilistic classifier in order to distinguish among nematode and non-nematode objects. Although these methods work powerful in order to distinguish between nematode and non-nematode objects, their False Rejection Rate (FRR) is significantly high. Moreover, optimization of the contours is a time consuming task, due to which these methods work very slow.

In this paper, we propose a skeleton-based analysis to detect and distinguish the individuals in population images with 83.7% True Acceptance Rate (TAR) and fast enough to be used in real-time applications. We also explain a possible application of this method in contour-based worm tracking algorithms in video sequences.

2. THE VISION IDEA

The proposed method is described in figure 2 in pseudo-code. The method consists of the following three parts:

2.1 Pre-processing

There are several methods in literature to obtain an image skeleton. We used a Gaussian smoothing filter with size
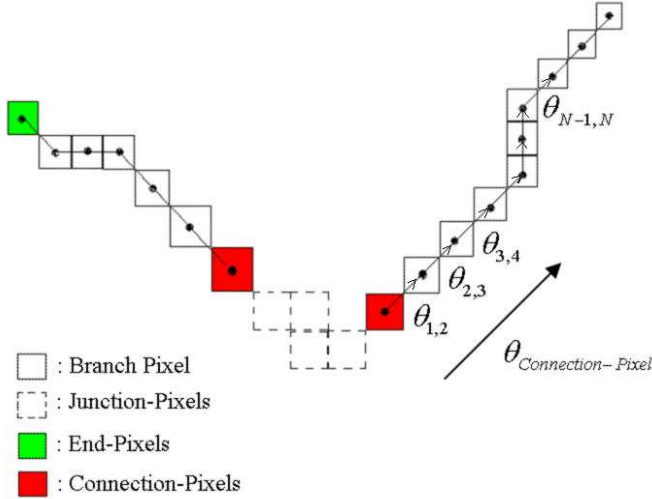
Figure 1: The angle calculation procedure of a Connection-Pixel. The red boxes, the dashed line boxes and the green boxes are Connection-Pixels, Junction-Pixels and End-Pixels, respectively. The θ_{ij} is the angle between the ith and j^{th} pixels in the same Connection-Branch.

20×20 and $\sigma = 1.0$ in order to blur the image. Subtraction of the original image from the blurred image results in detection of high frequency pixels (the edges). We use a global threshold calculated by the Otsu method to binarize the result. By applying a morphological closing we remove the small spots inside the objects. Then we produce the image skeleton using a morphological skeleton algorithm. Because of some spots inside the binary image, the skeleton has some extra branches which should be pruned in order to improve the algorithm efficiency. We use the following definition in the rest of the paper:

- Junction-Pixel: Pixels inside the skeleton with *more than* two neighbors.
- End-Pixel: Pixels inside the skeleton with *only one* neighbor.
- Connection-Pixel: Pixels inside the skeleton with only one neighbor after removing the Junction-Pixels.
- End-Branch: A branch with one End-Pixel and one Connection-Pixel in both sides.
- Connection-Branch: A branch with two Connection-Pixel in both sides.

2.2 Splitting skeleton into independent branches:

After obtaining a smooth skeleton, the skeleton is splitted into several branches by removing the Junction-Pixels which produces a combination of Connection-Branches and End-Branches. For each Connection-Pixel, an angle is calculated by averaging angles between sequential pixels for a user-defined number of pixels in the branch (N) starting from that Connection-Pixel. For example in figure 1, the angle of Connection-Pixel is:

$$\theta_{Connection-Pixel} = \frac{\theta_{1,2} + \theta_{2,3} + \theta_{3,4} + ... + \theta_{N-1,N}}{N-1} \quad (1)$$

The parameter N is important and should be assigned an appropriate value. For angle calculation, this parameter

Figure 2: An abstract explanation of our proposed method for detection and separation of individual worms in a population image.

not only must be big enough to avoid the influence of irregular sharp changes near the Connection-Pixels but it also should be small enough not to include the pixels far from the connection-pixel.

2.3 Merging branches for individual skeleton reconstruction:

For each End-Branch, an individual tree is assigned. The trees are grown up by attaching appropriate neighbor Connection-Branches. The following rules are used to construct a tree path:

1. The last branch of a path (r^{th} branch) is attached to k^{th} branch if firstly they have neighbor Connection-Pixels and secondly these Connection-Pixels angles satisfy:

$$|\theta_r - (180 + \theta_k)| \le \theta_{Th} \quad (2)$$

where θ_{Th} is defined by user.

2. The path growing stops when its last branch is an End-Branch or the neighbor branch can not satisfy the condition in (2).

To better understand, the procedure is explained by an example. There are three overlapped worms in figure.3. Refer to the definition, all branches except branch 4 are End-Branches. The algorithm starts from branch 1 by assigning an individual tree. The neighbors of this branch Connection-Pixel with $\theta_1 = 48^o$ are Connection-Pixels of branches 2,

3 and 4 with $\theta_2 = 126^o$, $\theta_3 = -57^o$ and $\theta_4 = -111^o$, respectively. For $\theta_{Th} = 50^o$, only θ_4 can satisfy the condition in (2). So branch 4 and $\theta_4{}'$ become the tree last branch and the tree last Connection-Pixel, respectively. The tree last Connection-Pixel ($\theta_4{}'$) has two neighbors: Connection-Pixels of branches 5 and 6 with $\theta_5 = 156^o$ and $\theta_6 = -39^o$, respectively. Because none of these angles satisfy the condition in (2), the tree path stops here. To conclude, the tree starting from the first End-Branch has only one path with two members: 1 and 4. The same procedure is executed for the other End-Branches.

3. EXPERIMENTAL RESULTS

The database used in this paper consists of 5 population images including 255 isolated and overlapped worms. We use three indications to judge the method:

- True Acceptance Rate (TAR): the number of correctly detected worms.
- False Acceptance Rate (FAR): the number of non-worm objects detected as worms.
- False Rejection Rate (FRR): the number of undetected true worms.

Table.1 indicates these rates for the utilized database. The method outcome has also been shown in figures 4 and 5. In both figures, the first row includes original image and the second row is the method result where green, yellow and red colors refer to the correct detected, incorrect detected and correct undetected worms, respectively. In our experience, the best result is obtained with $\theta_{Th} = 50^o$ and $N = 15$ pixels.

Table 1: The proposed method result for a database of 255 individuals with $\theta_{Th} = 50^o$ and $N = 15$ pixels.

	Percentage (Number)
True Acceptance Rate (TAR)	83.7% (232)
False Acceptance Rate (FAR)	7.9% (22)
False Rejection Rate (FRR)	8.4% (23)

4. CONCLUSION AND FUTURE WORK

In this paper, a skeleton based analysis is proposed for detection and separation of C.Elegans nematode worms in population images. The method first splits the skeleton obtained from a pre-processing step into several Connection- and End-Branches. Then it calculates an angle for each Connection-Pixel. Finally, an individual tree is assigned to each End-Branch. The tree is grown up based on angle analysis of its last branch and its neighbor branches. The algorithm chooses the appropriate neighbor branches with angle difference less than a user-defined threshold. Each path of the tree refers to a worm. Applying our method on a database of 255 nematode worms resulted in 83.7% accuracy for automatic detection. In addition to count the number of the worms by this method, its outcome can also be used as a first step of tracking of large number of nematodes in population video frames. In figure.6, the skeletons of two overlapped worms are detected and separated by our proposed method. A few pixels on each worm skeleton are chosen in order to divide the skeleton into several equal size segments. The perpendicular lines on skeleton in the chosen pixels are drawn. The intersection of these lines and the worm edge represent the worm contour points. These points can be used as an input material of contour-based tracking algorithm to track large number of specimens in population video sequences.
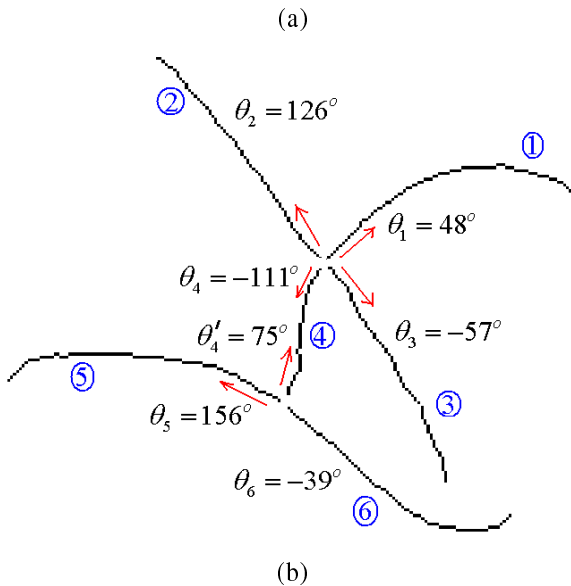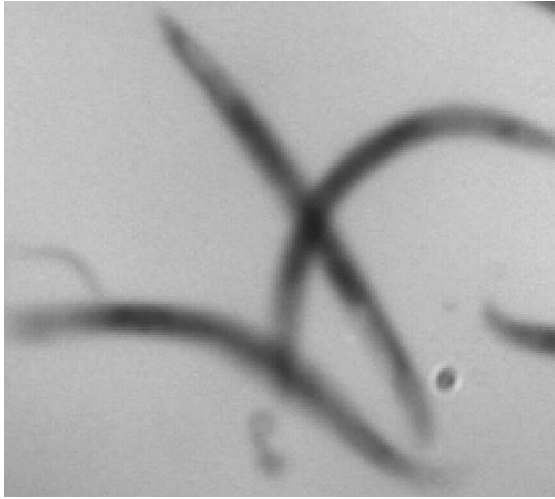
5. ACKNOWLEDGMENTS

(a)

(b)

Figure 3: A detail example to clarify the procedure: (a) original image (b) its smooth skeleton after removing Junction-Pixels. The Connection-Pixels angles are calculated with $N = 15$ pixels and $\theta_{Th} = 50^o$. Based on the method outcome, each of the paths 1-4, 2-3 and 5-6 are individual worms.

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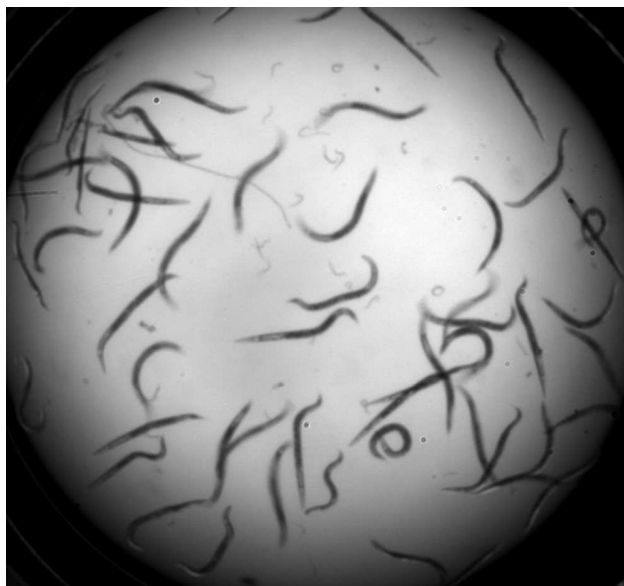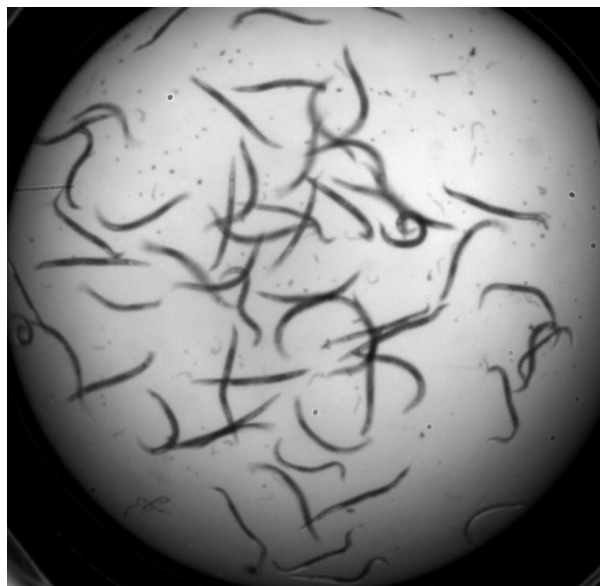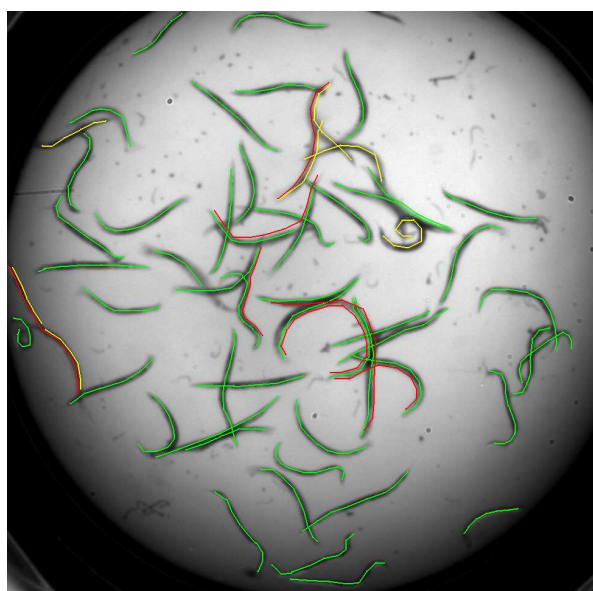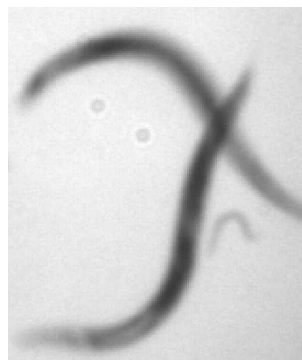
(a)
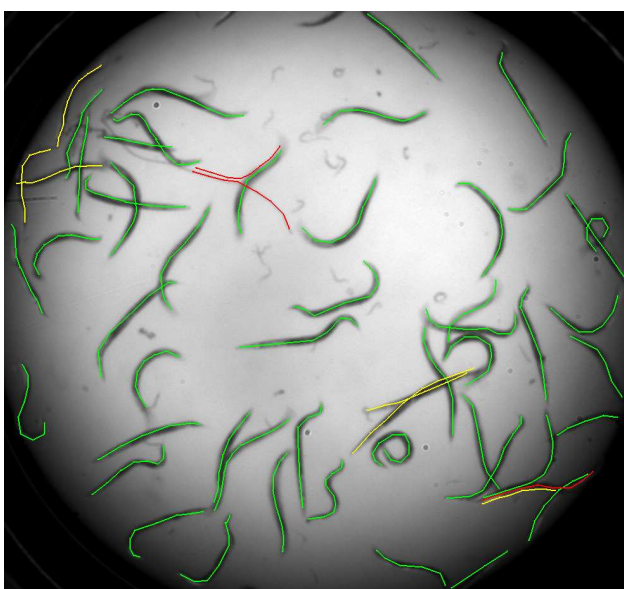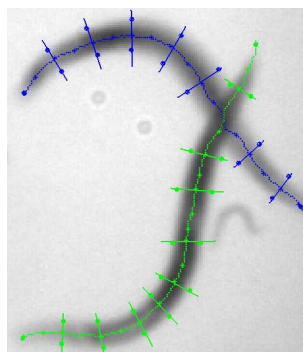

(b)

Figure 4: The first example: (a) original image (b) the final result. The green color represents correct detected worms related to TAR, the red color refers to incorrect detected worms related to FAR and the yellow color describes the correct undetected worms. The parameters N and θ_{Th} are 15 pixels and 50°, respectively.

(a)

(a)

(b)

(b)

(c)

Figure 6: The procedure to use the outcome of the proposed method in this paper for tracking a large number of worms in population video sequences: (a) The original image. (b) Choosing some pixels on each worm detected skeleton, drawing perpendicular lines and finding intersection of these lines and the worm edge. (c) The intersection points describing the worm contour can be used as input of a contour-based tracking algorithm.
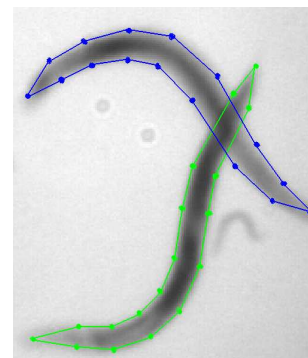
Figure 5: The second example: (a) original image (b) the final result. The green color represents correct detected worms related to TAR, the red color refers to incorrect detected worms related to FAR and the yellow color describes the correct undetected worms. The parameters N and θ_{Th} are 15 pixels and 50°, respectively.