Principles of automatic vision systems for tracking elongated microorganisms

Nikzad Babaii Rizvandi*[,1], Daniel Ochoa[1,2], Aleksandra Pizurica[1], Wilfried Philips[1]

[1]Department of Telecommunications and Information Processing, Image Processing and Interpretation Group, Gent University, St-Pietersnieuwstraat 41, B-9000 Gent, Belgium
[2]Faculty of Electrical and Computer Engineering, Computer Vision and Robotic Center, ESPOL University, Km 30.5 via perimetral, Guayaquil, Ecuador

In biotechnology research and development, studies on micro-organisms are necessary to evaluate the influence of chemical compounds in crops and to investigate, how environmental changes affect eco-systems or drug discovery procedures. These studies often involve visualizing large numbers of living organisms, counting and measuring them and tracking their movement. Currently, in most cases those organisms are imaged and analyzed individually in a time consuming process. In this paper, we address the existing problems in nematode imaging, detection and tracking, review recent research in this field, describe the advantages and disadvantages of the existing methods and finally propose our ideas to improve such vision systems. We focus on nematodes because their well-described nervous system, complete genome sequence and high developed digestive system are continuously raising interest in the research community for several years now and their shape configuration and motion types represent an interesting yet unsolved problem.

Keywords tracking elongated microorganisms, automatic vision system

1. Introduction

In biotechnology, studies on micro-organisms are a necessary step to assert the influence of chemical compounds on complex eco-systems. These studies often involve the visualization of large numbers of living organisms in order to find growth and reproductive patterns, describe the community composition and recently with the introduction of fluorescent markers measure the distribution of proteins and organ development. The nematoda phylum is one of the most primitive and abundant families of organisms that exist, nonetheless it shares many of the essential biological characteristics of human biology such as complete digestive and nervous systems. They are able to adapt to a free-living existence in most terrestrial and marine environments and parasitize in a wide number of plant and animal hosts. One of its members the Caenorhabditis Elegans used extensively as a model organism [1] has drawn increasing research and commercial interests since 1974 particularly in the fields of ecology, molecular and developmental biology. C. Elegans are unsegmented bilateral symmetric worms of varying thickness along their length, wide in the center and narrow near the head and tail. Its simple and featureless shape makes the incorporation of computer vision for detection and characterization a challenging task especially in cultures where numerous specimens interact with each other and the medium.

Although image processing tools have gained acceptance in daily lab work, the process is still labour intensive. Common software tools require marking points along the nematode body and then linear segments are automatically interpolated. The live-wire approach [2] facilitates contour drawing since by following the nematode body a line is attracted to their centerline. This semi-automatic approaches can effectively reduce the amount of time invested when dealing with single images containing few specimens but video-sequences and time-lapse images will require further automation given the higher amount of image data to be processed. As pointed out in [1] the need of high-throughput screening of bio-images to fully describe biological processes on a quantitative level is still very much in demand.

*Corresponding author: e-mail: nikzad@telin.ugent.be , Phone: +32 9 2643416

Recently, several integrated machine vision systems have been developed. The goal of such systems is tracking a significant numbers of individual organisms and measuring parameters such as size, mobility, shape features, or fluorescent marker distribution as a function of time.

2. Basic Principles and General Problems

Different kinds of equipment, media and methods can be used to grow nematodes depending on the particular research focus. For *C. Elegans* relatively standardized methods have been adopted over the years [2-3] making possible scientific results exchange. For instance, Escherichia coli 9001 and OP50 strains are common choices for providing food to nematodes in culture plates typically made of agar. During their life-cycle nematodes move freely on agar substrate and travel around their surroundings by bending their body in a sinusoidal movement. They can reach different parts of the plate, crawl over each other and occasionally plummet into the substrate.

Imaging living nematodes is done using inverted microscope and phase contrast technique as staining kills the animals. A high speed camera is required to avoid shadows produced when the sensor takes too long to integrate the signals -also known as motion blur- and consequently one pixel in the images will correspond to more than one point on the scene. The continuous improvement in microscopic techniques and imaging equipment allows capturing high quality video sequences but fundamental problems in the segmentation and recognition processes of individual nematodes remain open.

These problems can be classified in two broad categories: appearance and shape related. Let's consider appearance first, depending on the microscope settings nematodes can appear as a dark solid or semi-transparent line. Even individuals from the same sample might exhibit different intensity distributions. Optical density changes produce darker areas where internal organs become visible or at junctions when two or more specimens overlap. Besides nematode sections get out of focus while nematode dive into the agar. In fluorescent images the signal to noise ratio may be poor resulting in images where foreground and background are similar. Nematode's shape also pose problems resultant from the lack of salient contour features and complex motion patterns that prevent utilizing standard shape descriptors or simple mathematical models able to account for the complete range shape configurations. Even geometrical constrains on junction types found in overlapping situations are difficult to enforce given the random overlapping patterns. (Fig. 1)

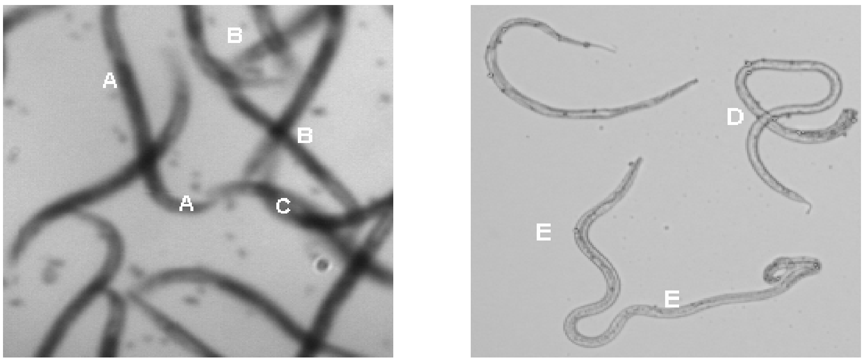

Fig. 1. Typical nematode images: note the lack of distinctive contour points. Also visible intensity variations along the same specimen (A,E), in overlapping regions (B), complex shape configurations (D) and heavy overlapping (C).

Despite the aforementioned issues the use of artificial vision to study nematodes has been the subject of numerous papers. The majority aimed at identifying and classifying isolated individuals under highly controlled conditions hence avoiding structural noise, artefacts and occlusion. In [4] images containing single nematodes are examined. After background correction, the image is thresholded and skeletonized, then contour curvature patterns are used to search for nematode's head and tail. By means of an interactive detection procedure, [5] proposed a method for nematode classification based on the morphological aspects of internal organs. In this work the stylet contour was used as input to a template matching algorithm that compared it with stylets of nematodes of known taxonomy. In a first step towards classifying *C.Elegans* behavioural phenotypes quantitatively, [6] identified motion patterns by means of a one-nematode tracking system, grey scale thresholding in addition to morphological operators and geometrical related features. In [7] nematode population analysis relies on carefully stacking basic image processing algorithms in a semi-automatic fashion.

More advance methods such as scale space principles were applied in elongated structures detection [8] but as in [9, 10] the efforts were focused on enhancing line evidence and its integration into objects was not pursued. Shape and image data integration is probably the most important issued in nematode and in general elongated object detection and characterization. In [18], video sequences of a single nematode are recorded using a tracking algorithm that controls the movement of a motorize stage keeping a nematode in the center of the frame. This approach simplifies recognition since the larger segmented object detected will always correspond to the nematode. Then, head and tail are found and 94 geometrical features defined and extracted from every frame. These features are input to the Random Forests classifier to identify up to 15 different mutant types. The database includes a total of 1597 individual nematode video sequences and the authors claim about 90% accuracy.

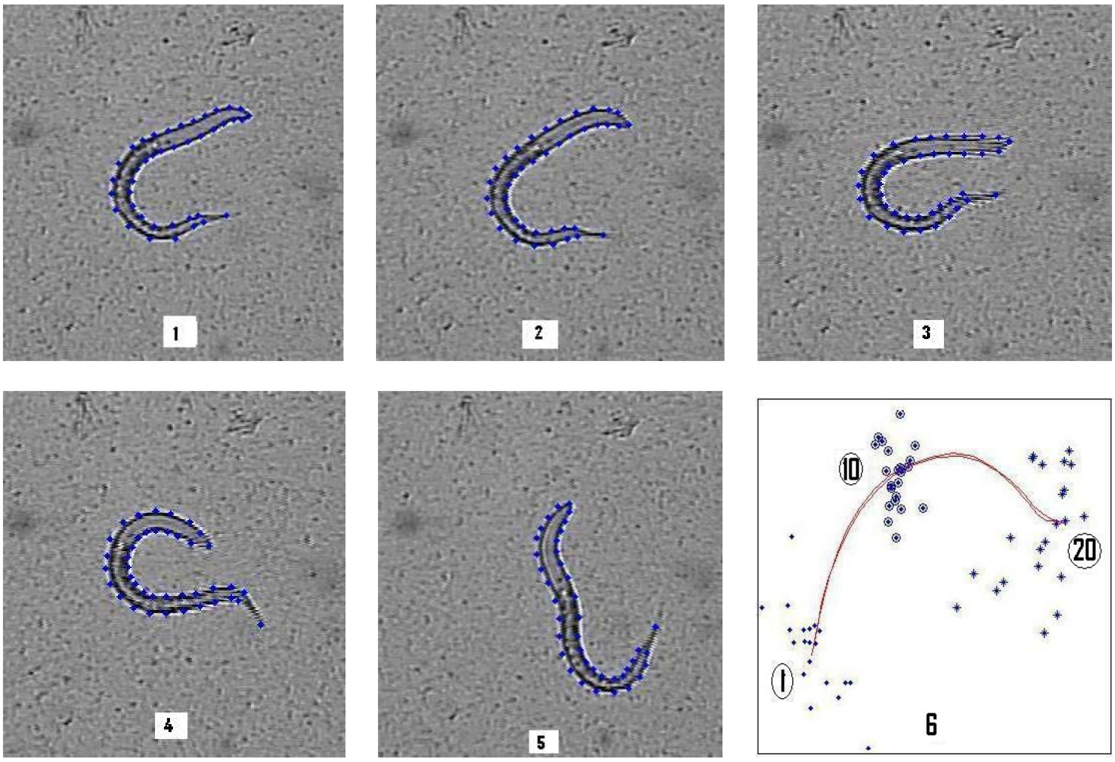

Fig. 2. (1-5) show a sequence of a C.elegans motions selected from 4.5 hours video sequence with 40 landmarks used in Active appearance model method [16]- thanks to prof. C.J. Taylor, university of Manchester. (6) shows the statistical model of landmarks number 1, 10 and 20 obtained from the training set images.

The preparation entailed in single nematode experiments could be substantially reduced by using images with multiple individuals. Unfortunately population images are more difficult to process and demand more complex algorithms to extract object evidence [30] reliably. This in turn increases the number of parameters making a balance between true and false detection rates a necessary condition [11]. Once such compromise is reached curves linking detected line points can be hypothesize and correlations between line profiles, scale and intensity exploited to detect nematodes. In our experiments the element different moment of neighbouring line segment proved the best feature for detecting isolated specimens [12]. This technique although useful for samples with a small amount of specimens is not suitable for discriminate nematodes in overlapping situations.

The utilization of deformable template models such as Active appearance model (AAM) and nonlinear estimation of the model's parameters using a central difference Kalman filter (CDKF) were examined in [15] and [17] for multiple nematode tracking. The model was build using 30 training images selected from 4.5 hours video sequence of an individual and then used to track a nematode 40 consecutive frames (Fig.2). This model was applied to establish the influence of certain genes in nematodes growth, development and ageing. A recently proposed method by some of us [13] also integrates shape and appearance modeling. In contrast to the previous method we resort to the energies of optimized active contours to discover common patterns in the image. The recovered shape information is then input to a probabilistic classifier and segmented contours are separated in nematode and non-nematode. Initialization is coped by using the ziplock model [14] that allows open contours and is less sensitive to get trapped in junctions. Nevertheless, new strategies are needed to reduce the number of contours to optimize. (Fig.3).

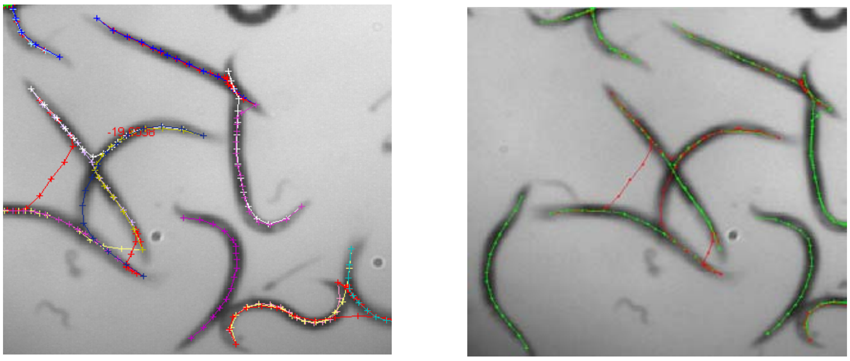

Fig. 3. Segmented contour after active contour optimisation (left). Classification results (right): nematode (green) and non-nematode (red) contours

3. Our Integrated Vision Idea

To provide consistent data to bio-researchers a vision system design must address varying imaging conditions, the lack of shape information and the non-rigid nature of nematodes. As mentioned in the previous section theses issues influence negatively the way nematodes -even in the same sample- appear and narrow the application of classic image processing techniques. In this section an integrated approach using a combination of multi-resolution and motion analysis that in corporate our past experience in various domains of microscopic biological and medical image analysis will be described.

Previous research work relies on assumptions such as high contrast between foreground and background or evenly distributed grey values on the nematode surface. Achieving such conditions demand additional work in experimental set up and in living samples are hardly guarantee anyway. We propose the application of multi-resolution approaches based on wavelet analysis [25,21] and steerable filters, especially in combination with probabilistic models based on Markov random fields [20,22,23] that have proved powerful tools for accurate image content modeling. Such techniques allow us to support image restoration -deblurring and denoising-, image segmentation, object tracking and co-localization analysis tasks within the same framework.

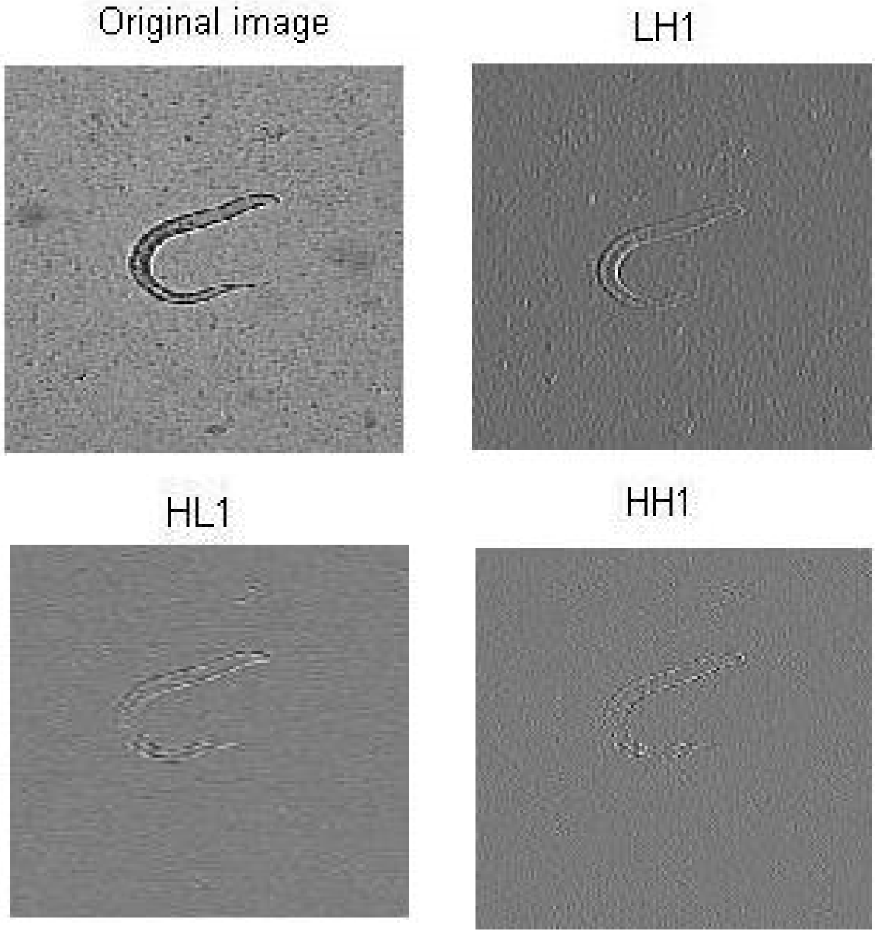

Fig. 4. Wavelet decomposition of a nematode image. Original image (left-up). Horizontal detail coefficients (right-up), vertical detail coefficients (left-bottom), diagonal detail coefficients (right-bottom).

Wavelet decomposition transforms images into two or more "detail" images and one "approximation" image, which shows the gross structure of the image (fig.4). In noise free images, most wavelet coefficients are nearly zero, except near image discontinuities, where the coefficients are typically large in magnitude. This property allows us to distinguish relevant image features from noise: small coefficients are usually related to noise and large ones to actual image content. This property is useful for noise suppression, where small coefficients are typically set to zero to remove noise without affecting the relevant image content. Another useful property is that wavelet coefficients representing actual image discontinuities display strong inter- and intra-scale statistical dependencies. This property is often exploited in noise suppression, segmentation and compression by using Hidden Markov Tree (HMT) [20] and related Markov Random Field (MRF) [22] models. We believe that using MRF models to capture the spatial properties of nematodes shape, e.g., the fact that they are continuous curves, will help to reduce nematode contour fragmentation. We propose to extend multi-resolution models by using the intra-band local line orientation, as determined within each wavelet detail image that must be consistent with the orientation in the other wavelet detail images. Similarly, the inter-band energy distribution of the wavelet coefficients must also be consistent with this orientation. These two types of consistency only hold for wavelet coefficients due to image features and not for those caused by noise. Therefore, detecting the degree of consistency can significantly improve nematode segmentation.

A related approach is the development of algorithms based on steerable filters. Steerable filter decompositions are similar to those based on wavelets but with a higher orientation selectivity. The innovative ideas exploited in wavelet based techniques, and in particular the ones discussed above, can be combined with steerable filters as well. Since steerable filters provide much better orientation sensitivity, their use can potentially increase the accuracy of our nematode evidence consistency approach. This will be done by extending our past research on microscopic image deblurring [24].

Nematode evidence integration into meaningful line segments problem will be tackled by combining the active contour and active appearance model (AAM) approaches. An AAM model is constructed from a group of input images and a set of landmarks located on the object [27]. Its aim is to match the trained model to a new instance of the object. To simplify model building we will study the application of active contour energies to capture common patterns to enable better strategies for automatic landmark point location. Experiments will compare open, close, parametric and geometric active contour models to measure which one provides the most useful representation. Once the landmark points are located modelling the possible non-linear shape configurations requires alternative methods to linear principal component analysis (PCA), since declining the model flexibility to reduce parameter space fails to accurately model nematodes. We propose to extend research lines in independent component analysis (ICA) [28] and kernel-based principal component analysis (KPCA) [29] to improve nematode's shape modelling.

Regarding tracking, existing methods are mostly based on a combination of still-image segmentation and motion estimation. Since our goal is to develop an integrated multi-resolution method, we plan to perform motion estimation in the wavelet or steerable filter domain as is the case in some state-of-the-art wavelet-based video coders [19]. An effective approach is to model motion using dense motion fields, rather than a single motion vector per block. Another idea is modelling the reliability of the motion vector field in each position of the image. It is well-known that near edges only one component of a motion vector can be reliably estimated using local information (the aperture effect), whereas in most image regions even that is not possible. So our objective is to estimate the accuracy from local features [26].

4. Conclusion

This chapter describes the basic principles and general problems of nematode vision, reviews the existing vision systems for nematodes and finally proposes a novel idea for a vision system based on the state-of-the-art methods. We envision a wavelet-based nematode detection system. Wavelets have been used extensively for image denoising, segmentation and line detection, but not applied to the specific problem of nematode detection yet. Another important segment of the proposed system is a modified active appearance model using kernel principle component analysis (KPCA) to overcome the non-linear nature of the nematode movement. We discussed two possible approaches for tracking stage: (1) to segment the object and then use motion estimation algorithm, such as Kalman filter, to track the nematode in a video sequence and (2) an integrated approach in the wavelet or steerable filter domain. The proposed integerated vision system concentrates on the problems in tracking a single nematode. Simultaneous tracking of multiple nematodes will require solving additional practical problems, such as crossing of two or more nematodes. This problem and exprimental evaluation of the envisioned system is a topic of our ongoing research.

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