Detection of Individual Specimens in Populations Using Contour Energies

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Abstract. In this paper we study how shape information encoded in contour energy components values can be used for detection of microscopic organisms in population images. We proposed features based on shape and geometrical statistical data obtained from samples of optimized contour lines integrated in the framework of Bayesian inference for recognition of individual specimens. Compared with common geometric features the results show that patterns present in the image allow better detection of a considerable amount of individuals even in cluttered regions when sufficient shape information is retained. Therefore providing an alternative to building a specific shape model or imposing specific constrains on the interaction of overlapping objects.

Keywords: recognition, feature extraction, statistical shape analysis.

1 Introduction

An important tool for biotechnology research and development is the study of populations at molecular, biochemical and microbiological levels. However, to track their development and evolution non-destructive protocols are required to keep individuals in a suitable environment. The right conditions allow continuous examination and data collection that from a statistically meaningful number of specimens provide support for a wide variety of experiments. The length, width and location of microscopic specimens in a sample are strongly related to population parameters such as feeding behavior, rate of growth, biomass, maturity index and other time-related metrics.

Population images characterized by sample variation, structural noise and clutter pose a challenging problem for recognition algorithms [1]. These issues alter negatively the estimated measurements, for instance when parts of the detected object are out of focus, two or more individuals can be mistakenly counted as one or artifacts in the sample resembles the shape of specimens of interest. A similar condition occurs in tracking applications when continuous identification of a given individual, while interacting with others of the same or different phylum is required. Nevertheless the increasing amount of digital image data in micro-biological studies prompts the need of reliable image analysis systems to produce precise and reproducible quantitative results.

The nematodes are one of the most common family of animals; they are ubiquitous in fresh water, marine and terrestrial eco-systems. As a result nematodes populations had become useful bio-indicator for environmental evaluation, disease expressions in crops, pesticide treatments, etc. A member of the specie, the C. Elegants nematode is widely applied in research in genetics, agriculture and marine biology. This microorganism has complete digestive and nervous systems, a known genome sequence and is sensitive to variable environmental conditions.

Intensity thresholding and binary skeletonization followed by contour curvature pattern matching were used in images containing a single nematode to identify the head and tail of the specimen [2]. To classify C.Elegans behavioral phenotypes in [3] motion patterns are identified by means of a one-nematode tracking system, morphological operators and geometrical related features. The advantages of scale space principles were demonstrated on nematode populations in [4] and anisotropic diffusion is proposed to improve the response of a line detection algorithm; but recognition of single specimens was not performed.

In [8] nematode population analysis relies on well-known image processing techniques namely intensity thresholding followed by filling, drawing and measuring operations in a semi-automatic fashion. However sample preparation was carefully done to place specimens apart from each other to prevent overlapping. Combining several image processing techniques when dealing with biological populations specimens increase the complexity of finding a set of good parameters and consequently reduce the scope of possible applications.

Daily lab work is mostly manual, after the sample image is captured a biologist define points along the specimen, then line segments are drawn and measurement taken. User friendly approaches like live-wire [5] can ease the process as while pointing over the nematode surface a line segment is pulled towards the nematode centerline. Though in cluttered regions line evidence vanishes and manual corrections are eventually required. Considering that a data set usually consists of massive amounts of image data with easily hundreds of specimens, such repetitive task entails high probabilities of inter-observer variations and consequently unreliable data.

Given the characteristics of these images, extracting reliable shape information for object identification with a restricted amount of image data, overlapping, and structural noise pose a difficult task. Certainly, the need of high-throughput screening of bio-images to fully describe biological processes on a quantitative level is still very much in demand [6]. Unless effective recognition takes place before any post-processing procedure the utilization of artificial vision software for estimating statistical data from population samples [7] will not be able to provide with accurate measurements to scientists.

As an alternative to past efforts focused at deriving shape models from a set of single object images using evenly distributed feature points [14]. We propose recover shape information by examining the energies of sample optimized active contours from a population image. In order to assert the efficiency of such approach we compare them with geometrical measurements. Our aim is to prove that patterns extracted from sample contours can lead to recognition of individual specimens in still images even in the presence of the aforementioned problems.

This paper is organized as follows. In section 2 the active contour approach is discussed. Shape features of detected nematodes are proposed and used for classification in Section 3. Comparative results are shown in Section 4; finally conclusions and future work is presented in Section 5.

2   Segmentation Using Active Contours

Nematodes are elongated structures of slightly varying thickness along their length, wide in the center and narrow near both ends. Contrary to one might think its simple shape makes segmentation process a complex task in population images because nematodes interact with the culture medium and other specimens in the sample. Nematodes lie freely on agar substrate and explore their surroundings by bending their body. While foraging, nematodes run over different parts of the image, crawl on top of each other and occasionally dive into the substrate. This behaviour leads to potential issues in segmentation because substantial variations in shape and appearance are observed in population images.

Nematodes exhibit different intensity level distributions either between individuals or groups when image background is non-homogeneous. Darker areas appear every time internal organs become visible or at junctions when two or more specimens overlap. Some parts get blurred as they get temporarily out of focus when diving into the sustrate. Regarding shape, the lack of contour features and complex motion patterns prevent using simple shape descriptors or building models able to account for the whole range shape configurations. These two characteristics also make difficult to find a set of geometrical constrains that can illustrate all the junction types found in overlapping situations Fig. 1.

Under these conditions, thresholding techniques commonly used in images of isolated specimens fail to provide a reliable segmentation. Approaches based on differential geometry [11] can handle better the intensity variation, but a trade off between the image-content coverage and conciseness [12] is needed to set appropriate parameter values. Statistical tests on hypothetical center-line and background regions at every pixel locations as proposed in [23] rely on having enough local line evidence, which precisely disappear at junctions where saddle regions form. The inherent disadvantages of the aforementioned techniques allow in practice to obtain only a set of unconnected points hopefully the majority located on the traversal axis of some of the nematodes present in the image.

Line grouping based on graph search and optimisation techniques enforcing line continuity and smoothness were applied to integrate line evidence [13,23], but segmentation of objects based on linear segments requires relevant local segments configurations that capture objects shape characteristics [22]. Shape modelling assuming evenly distributed landmark points along nematode body proved a complex issue, although non-linear systems had been devised [10] the complete range of nematode body configurations is still far from being model. Spatial arrangement of feature points at different scales were exploited in [15] to search for regions of high probability of containing a rigid wiry object in different cluttered environments, yet in populations clutter is mostly caused by nematode themselves.

Fig. 1. Left: Nematodes in a population image. Center: Structural noise produced by internal organs, and overlapping. Right: Non-homogenous background cause differences in appearance.

In this paper we propose the utilization of active contours energies to capture relevant statistical shape information for recognition applied to nematode detection in population images. Active contours introduced by Kass with a model called snake [16] has drawn attention due to their performance in various problems. Segmentation and shape modeling in single images proved effective by integrating region-based information, stochastic approaches and appropriate shape constrains [17, 18].

Active contours combine image data and shape modeling through the definition of a linear energy function consisting of two terms: a data-driven component (external energy), which depends on the image data, and a smoothness-driven component (internal energy) which enforces smoothness along the contour.

$$E_{contour} = \lambda_1 \cdot E_{int} + \lambda_2 \cdot E_{ext} \tag{1}$$

The internal energy can be decomposed further into tension and bending energies, they report higher values as the contour stretches or bends during the optimization process. The goal is to minimize the total energy iteratively using gradient descent techniques as energies components balance each other.

$$E_{int} = \int_0^S e_t(s) + e_b(s)ds , \qquad E_{ext} = \int_0^S e_{ext}(s)ds \tag{2}$$

The proposed approach is based on the idea that given convergence of the active contours mostly data-driven, appearance and geometrical data can be recovered from the resulting energy component value distribution. Contrary to other works that tried to embed partial shape information to guide the evolution of the contour [21], we consider the analysis of energy based derived features a natural way to explore the range of possible nematode shape configurations in a set of population images without having to build an specific model or making explicit constrains about objects interaction [19]. We leave to the active contour optimization process the task of locating salient linear structures and focus on exploiting the distribution of energy values for recognition of those contours corresponding to nematodes.

For segmentation we used ziplock snake [20], this active contour model is designed to deal with open contours. Given a pair of fixed end points optimization is

carried out from them towards the center of the contour using in every step a increasing number of control points. This procedure is intended to raise the probability of accurate segmentation by progressively locating control points on the object surface. They can encode shape information explicitly [21] and provide faster convergence than geodesic snakes.

It is important to point out that as in any deterministic active contour formulation there are situations in which convergence tends to fail. For instance in the presence of sharp turns, self-occlusion or in very low contrast regions. Nevertheless as long as the number of correct classified contours represent a valid sample of the population we can obtain meaningful data for bio-researchers. In the context of living specimens we should expect that eventually every individual will have the possibility of match with a nicely converged contour.

For our experiments, the tension energy e_t was defined as the point distance distribution, the bending energy e_b calculated by means of a discrete approximation of the local curvature and a normalized version of the intensity image was employed as energy field e_{ext}.

$$e_{ext}\alpha\,I(x, y), \qquad e_t = \sqrt{\dot{x}^2 + \dot{y}^2}, \qquad e_b = \left|\frac{(\dot{x}\cdot\ddot{y} - \ddot{x}\cdot\dot{y})}{(\dot{x}^2 + \dot{y}^2)^{3/2}}\right| \qquad (3)$$

The main bottleneck in the automated use of ziplock snakes is the need for specifying matching end points for a contour. The absence of shape salient features in head and tail nematode sections prevents building a reliable matching table. The only option is to examine all possible combination of points, but this can lead to a combinatorial explosion of the search space. In this context we devised two criteria to constrain the number of contours to analyze:

- Matching end points within a neighborhood of size proportional to the expected nematode length,
- Matching end points connected by path showing consistent line evidence.

Fig. 2 depicts initial contours generated after applying the both criteria. In the first case the nematode length was derived from a sample nematode, in the second case the raw response of a line detector [24] was used to look for line evidence between end points. Any path between a pair of end points consisting of non-zero values was considered valid and allows the initialization of a contour.

Once the contours had converged, we observe different situations regarding their structure:

- The contour can be located entirely on a single nematode.
- The contour sections correspond to different nematodes.
- Part of the contour lies on the image background.

The first case requires both end points to be located on the same object, occurs when the specimen is isolated or the energy optimization is able to overcome overlapping regions. The second type of contour appears when a contour spreads among overlapping nematodes while fitting a smooth curve between its end points. If

the smoothness constrain can not be enforce some contour sections might rest on the image background.

In the following we will refer to contours located on single nematode as nematode contours and the remaining cases as non-nematode contours. Our interest is to extract nematode contours reliably, but as can be seen in Fig. 2. there is no simple way to distinguish them without additional processing steps and the inconvenient problems mentioned previously.   Hence the suggested solution is presented in the following section.
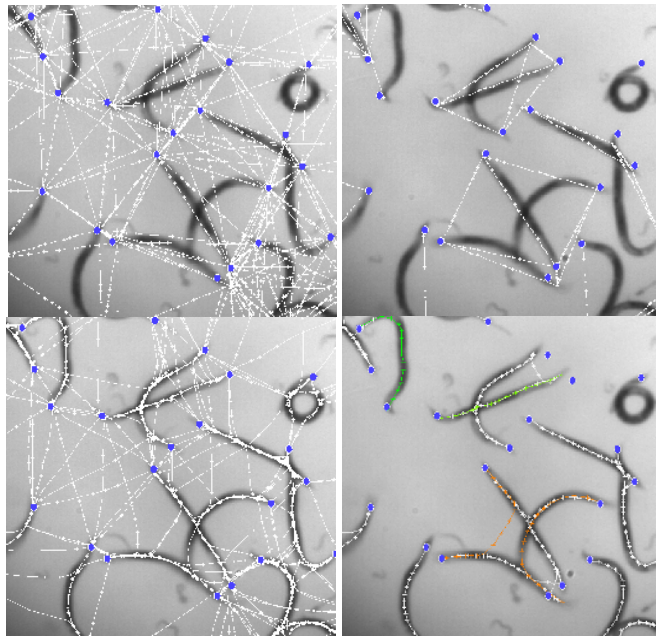

Fig. 2. Contours (white) from end points (blue) matching criteria. Left column: expected length. Right column: line evidence. First row: before convergence. Second row: after convergence. Right bottom: Examples of nematode (green) and non-nematode (orange) contour classes.

3   Detection of Specimens Using Energy Features

The goal of our experiments is to explore the feasibility of classifying a given contour in a corresponding nematode w_n or non-nematode w_t classes. Let C be the set of contours $\{c_1,...,c_m\}$ generated after the convergence process and define a contour c as a sequence of n control points $(x_1,...,x_n$). Two types of shape measurements based on the three relations (length, curvature and line evidence) encapsulated in the energy terms are defined.

The expected point energy M_e captures the average value of a given energy term e along the contour:

$$M_{c,e} = \bar{e}_c \quad , e \in \{e_t, e_b, e_{ext}\} \tag{4}$$

and the point sequence energy S_e integrates the control point's energy in a vector providing evidence about the effect that different shape and appearance configurations have on the individual contour components:

$$S_{c,e} = (e^c_{x_1}, \ldots, e^c_{x_n}) \quad , e \in \{e_t, e_b, e_{ext}\} \tag{5}$$

The distributions of these energy based feature values allows us to study the similarity between contours belonging to objects of interest and their properties. It seems reasonable to expect that the energy configuration space should display clusters in regions linked to objects of consistent shape and appearance.

The relevance of using active contours and their associated energies becomes manifest when comparing contours after convergence. In background regions, control points are collinear and equidistant, therefore M_e features should report rather fixed values. For nematode contours, control point spatial distribution is not homogeneous because their location is determined by the foreground image data and body geometrical configuration. Since at some degree they look alike and share similar movement behavior a suitable set of S_e features values could capture such limited configuration space.

Other patterns can be deduced, but it is unlikely that features derived from any individual energy term will provide by itself a reliable recognition outcome. The combination of energy based features in a statistical framework is proposed to measure their discriminative power. To that aim the Bayes rule was applied to classify contours as nematode or non-nematode. The ratio of the a posteriori probabilities of nematode to non-nematode classes given the values of an energy based feature set was defined as discriminant function.

The prior probabilities were regarded homogeneous to test the effectiveness of the proposed features, however they can be modeled for instance by the distribution of control point distances to the nearest end point or by the distribution of line evidence. This reduces the discriminant function to the ratio of the probabilities of feature values given that a contour is assigned to a particular class. Assuming independence between energy terms and control point locations theses distributions can be readily defined as the product of the probabilities of the feature set elements given a class $w \in \{w_n, w_t\}$:

$$P(M_{c,e} \mid w) = \prod_e P(\bar{e}_c \mid w), e \subseteq \{e_t, e_b, e_{ext}\} \tag{6}$$

$$P(S_{c,e} \mid w) = \prod_e \prod_x P(e^c_x \mid w), e \subseteq \{e_t, e_b, e_{ext}\} \tag{7}$$

Finally, the computational cost for contour classification in a population image depends on the size of C, the feature type selected and the number of energy terms included. In the case of S_e there is no extra cost because their components are the terms of $E_{contour}$, M_e calculations requires an additional step to calculate the associated average.

4   Experimental Evaluation

The proposed methodology was evaluated on a set of high resolution time-lapse images depicting populations of adult nematodes with approximately 200 specimens. The end point set was extracted from ground truth images and straight initial contours placed between pairs of matching points according to the criteria presented in section 2. Both contour sets with 903 and 1684 elements, each having 16 control points, were optimized until convergence. To estimate the conditional probability distributions we built a training set of 50 randomly selected nematodes and non-nematode contours. Given the non-gaussian nature of $P(M_e|w)$ and $P(S_e|w)$ data we fitted them using weibull and gamma probability density functions respectively to extract the distribution parameters.

   The features derived from the expected point energy and the point sequence energy definitions, comprised all the possible combinations of energy terms. Every feature type was evaluated separately and combined totaling 21 energy based features. For completeness we included also the total contour energy $E_{contour}$. We additionally performed energy based feature classification considering different number of control points. To do that an increasing number of control points on both ends of every contour was gradually discarded.

   To assert the performance of the proposed energy based features we compared them to geometrical features used in previous work on nematode classification [3]. They include: the contour length Len, the summation of signed distance from the end points to the contour's centroid that provides a measure of symmetry Sym, a compactness Cmp metric calculated as the ratio between the contour length and its eccentricity, and the angle change rate Acr computed from the summation of the difference in angles between contour segments normalized by the length and number of control points. We tested them separately and combined using the same probabilistic framework described in section 3.

   Table 1. summarizes the classification results, it shows the true positive Tp rate, the false positive Fp rate, and the distance D to perfect detection corresponding to best performance for every feature type. In the case of energy based features the first

Table 1. Best classification results for energy and non-energy based feature combinations

	Line Evidence			Expected length		
	D	Tp	Fp	D	Tp	Fp
$S^{16}_{(e_t, e_b, e_{ext})}$	0.263	0.884	0.236	0.137	0.911	0.104
$M^{10}_{(e_t, e_{ext})}$	0.406	0.614	0.125	0.227	0.800	0.108
$M + S^{12}_{(e_t, e_{ext})}$	0.543	0.467	0.106	0.398	0.604	0.044
$Len + Sym + Acr$	0.479	0.924	0.473	0.352	0.901	0.338
$E_{contour}$	0.747	0.924	0.743	0.736	0.923	0.732

column also specifies the energy terms included and the amount of control points. The proposed energy based features consistently show a better trade off between true and false detection rates compared to other features. Though in combination the true positive detection drops it is still comparable with non-energy based features that despite of detecting most nematode contours have a high rate of false detections. The total contour energy $E_{contour}$ performed poorly.

Point sequence features discriminative power increases as more control points are added while for expected point energy features results improves when this number decreases. This is indicative that nematode and non-nematode contour classes have similar average energy value distributions and only when the contour's central part is analyzed the difference is large enough to allow reliable classification. A possible explanation relies on the fact that nematodes central area is the less flexible part of their body so contour variations become prominent if we use only the central control points. Regarding the two search spaces we noticed that results improve as we include more initial contours since we have more possibilities of segmenting all the nematodes contained in the sample.
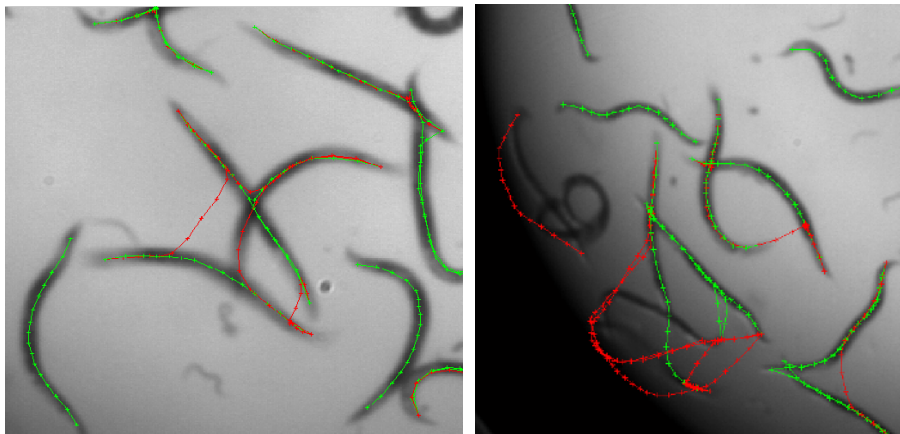

Fig. 3. Classification results for nematode (green) and non-nematode contours (red) some non-nematode contours were remove to improve visibility

The results showed that the single most discriminating energy term for M_e , S_e and $M_e + S_e$ features is the tension energy term e_t, the spatial distribution of control points appears to capture nematode evidence accurately. This observation is explained in terms of the relations between energy terms during optimization. Since in our image set nematodes show lower external energy e_{ext} values near the center, control points tend to gather in that area however as they move e_t increases in the vicinity of contour ends and pulls them in the opposite direction. Therefore, the distance between control points varies depending on the regions they are located, in our specimens these regions correspond to nematode appearance features. It must be noted that only by combining several energy terms the false positive rate can be consistently reduced. As expected bending energy e_b allow us to filter out contours with sharp turns and the

external energy e_{ext}, those with spatial intensity distribution too different from those found in the population Fig. 3.

Nematode contour misclassification occurs when appearance information is lost or in the presence of an unusual shape configuration. The first case includes nematodes close to the petri dish border where lightning conditions reduce the contrast between foreground and background. The other case is frequently the result of optical distortion produced by the microscope lens. Non-nematode contours can be mistakenly classified when most of their control points converge towards a real nematode, for instance in the presence of parallel nematodes very close to each other, or when in heavy overlapping regions a contour manages to run over parts of several objects and still resemble a real nematode Fig. 4.
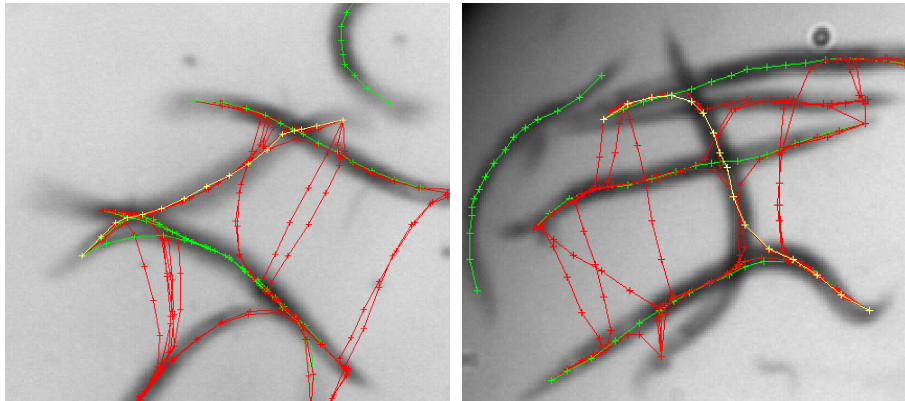

Fig. 4. Misclassification examples (yellow). Right: nematode contour affected by blur. Left: non-nematode contour partially running over different nematodes in overlapping region.

The change of relative optical density at junction constitutes the main source of structural noise. The resulting darker areas affect negatively the spatial distribution of control points during the optimization process and hence the recovered energy values. The more occluded is a nematode the less its discriminant function value, nevertheless correct detection of a number of nematodes in overlapping regions is feasible when enough shape information is retained. We also noticed that nematode contours sharing a end point with wrongly detected contours have a consistently higher discriminant function value, this relation could be used to improve detection results further but has not explored yet in these experiments.

5   Conclusions

A set of features for detection of individual nematodes in population has been proposed. The resultant patterns from a set of optimized contours proved a valid source of shape evidence for recognition of specimens in difficult scenarios. Detection rates allowed us to reject most non-nematode contour while keeping a significant number of correct detected nematodes.

The proposed approach differ from existing shape modeling approaches where feature points are manually located on salient regions on individual object to build linear and non-linear shape model. We use the evolution of active contour models to capture object statistics therefore constraining the range of possible appearance and geometrical configurations to those present in the current sample set.

Features based on average and local contour energy component distributions were tested on manually segmented images in the framework of Bayesian inference. Experimental results with two different contour initialization strategies show that energies based features provide better detection rates that geometrical based features commonly applied in image processing of biological samples. In particular energy term combination displayed a consistent performance for true nematode detection. When nematode and non-nematode contours have similar average feature values the results can be improved if only the central region of the contour is evaluated which is consequent with the morphological characteristic of these specimens captured during the optimization process.

Despite the limitations of active contours to converge correctly in low contrast regions or in the vicinity of sharp corners we found out that recognition is still feasible if a sufficient amount of shape information is retained even in overlapping regions. Further improvement in detection rates could be achieved if interactions between classified contours and prior knowledge about line evidence are included however this work is out of the scope of this paper. We let for future work extending our findings to video sequences for tracking moving nematodes in occlusion situations.

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