**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 allo w 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 variatio n, 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.

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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 kno wn geno me

sequence and is sensitive to variable environmental conditio ns.

Intensity thresholding and binary skeletonization followed by contour curvature

pattern matchin g 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 perfo rmed.

In [8] nematode population analysis relies on well-known image processing

techniques namely intensity thresholding followed b y 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 bio logist

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. Tho ugh 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 variation s and conseq uently 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 describ e bio logical processes on a quantitative level is still very

much in demand [6]. Unless effective recognition takes place before any p ost-

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.

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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

probab ility of containing a rigid wiry object in different cluttered environments, yet in

populations clutter is mostly caused by nematode themselves.







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**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 shap e information for recognition applied to nematode d etection 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 co mbine 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*

*=*

·

*E*

*+*

·

*E*

(1)

*co nt o ur*

*1*

i nt

*2*

*ext*

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.

*S*

*S*

*E*

*=*

*e*

*(s)*

*+*

*e*

*(s)ds*

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*E*

*=*

*e*

*(s)ds*

(2)

i nt

*t*

*b*

*e x t*

*e x t*

0

0

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 mo del 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

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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

probab ility 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 p oint 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

sho uld expect that eventually every individual will have the possibility of match with

a nicely converged contour.

For our experiments, the tension energy

*e*

was defined as the point distance

*t*

distribution, the bending energy

*e*

calculated by means of a discrete approximation of

*b*

the local curvature and a normalized version of the intensity image was employed as

energy field

*e*

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*e xt*

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*x*

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*y*

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*x*

*y*

*)*

*a*

*e*

*I(x,*

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*e*

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*x*

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*y*

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*e*

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(3)

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*x*

*+*

*y*

*)*

*e x t*

*t*

*b*

2

2

3

/

2

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 sectio ns 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

nematod e length,

•

Matching end points connected by path showing consistent line evidence.

Fig. 2 depicts initial conto urs 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.

•

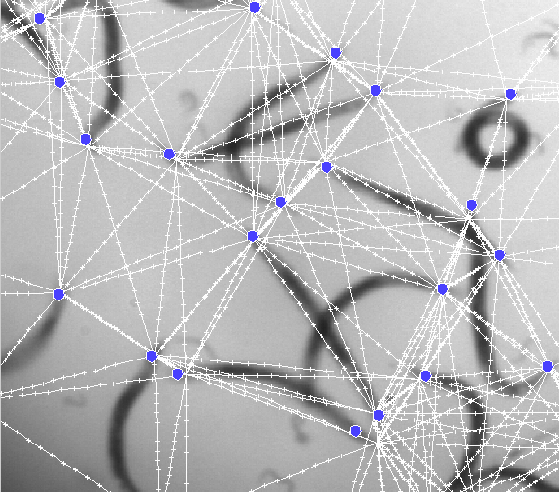
Part of the contour lies on the image background.

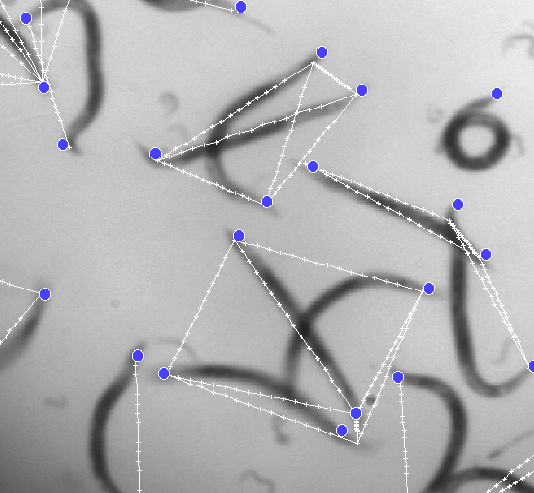
The first case requires both end points to be located on the same object, occurs

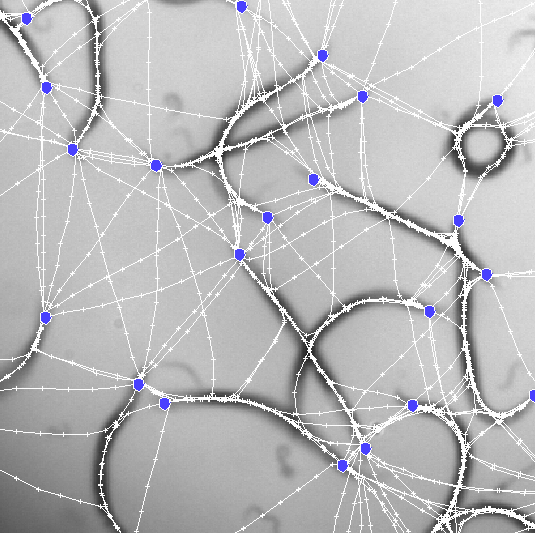
when the specimen is isolated or the energy optimization is able to overcome

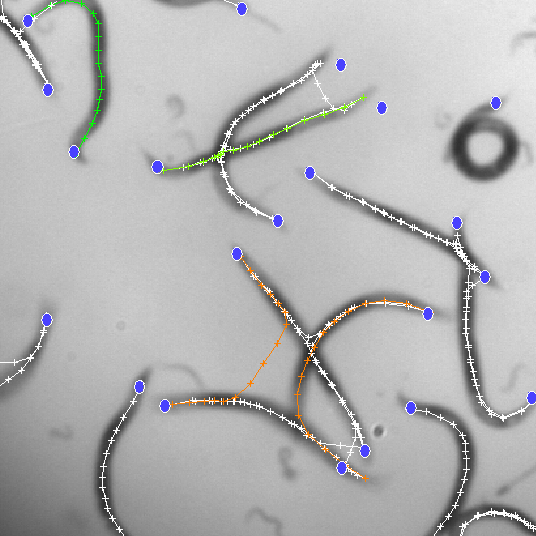
overlapping regions. The second type of contour appears when a contour spreads

among overlapping nematodes while fitting a smooth curve between its end points. If









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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*

or non-nematode

*w*

classes. Let

*C*

be the set of

*n*

*t*

contours

*{c*

*,...,c*

*}*

generated after the convergence process and define a contour

*c*

as a

*1*

*m*

sequence of

*n*

control points (

*x*

*,...,x*

)

*.*

Two types of shape measurements based on

*1*

*n*

the three relations (length, curvature and line evidence) encapsulated in the energy

terms are defined.

The expected point energy

*M*

captures the average value of a given energy term

*e*

*e*

along the contour:

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{}

*M*

*=*

*e*

,

*e*

*e*

,

*e*

,

*e*

(4)

*c*

,

*e*

*c*

*t*

*b*

*ext*

and the point sequence energy

*S*

integrates the control point’s energy in a vector

*e*

providing evidence about the effect that different shape and appearance

configuratio ns have on the individual contour components:

{}

*S*

*=*

*(e*

,...,

*e*

*)*

,

*e*

*e*

,

*e*

,

*e*

*c*

*c*

(5)

*x*

*c*

,

*e*

*x*

*t*

*b*

*ext*

*n*

1

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*

features should report rather fixed

*e*

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*

features values could capture such limited

*e*

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 o r 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 prob abilities were regarded homo geneous 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 d iscriminant functio n to the ratio of the prob abilities 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*

{

*w*

,

*w*

}

:

*n*

*t*

{}

*P(M*

*|*

*w)*

*=*

*P(*

*e*

*|*

*w)*

,

*e*

*e*

,

*e*

,

*e*

(6)

*c*

,

*e*

*c*

*t*

*b*

*e x t*

*e*

{}

*P(S*

*|*

*w)*

*=*

*P(e*

*|*

*w)*

,

*e*

*e*

,

*e*

,

*e*

(7)

*c*

*c*

,

*e*

*x*

*t*

*b*

*e x t*

*e*

*x*

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*

there is no extra cost because their components are the

*e*

terms of

*E*

,

*M*

calculations requires an additional step to calculate the associated

*c ont ou r*

*e*

average.

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**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*

*|w)*

and

*P(S*

*|w)*

data we fitted them using

*e*

*e*

weibull and gamma probab ility density functio ns respectively to extract the

distribution parameters.

The features derived from

the expected point energy

and the point sequence energy

definitio ns,

comprised all the possible combinations of energy terms. Every feature

type was

evaluated separately and combined totaling 21 energy based features. For

completeness we includ ed also the total contour energy

*E*

*.*

We additionally

*c ont ou r*

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 geo metrical 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

probab ilistic framework d escrib ed 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

*,*

*,*

0.263 0.884 0.236 0.137 0.911 0.104

*(e*

*e*

*e*

)

*t*

*b*

*e xt*

*M*

1 0

*,*

0.406 0.614 0.125 0.227 0.800 0.108

*( e*

*e*

)

*t*

*ext*

*M*

*+*

*S*

*,*

0.543 0.467 0.106 0.398 0.604 0.044

1 2

*(e*

*e*

)

*t*

*ex t*

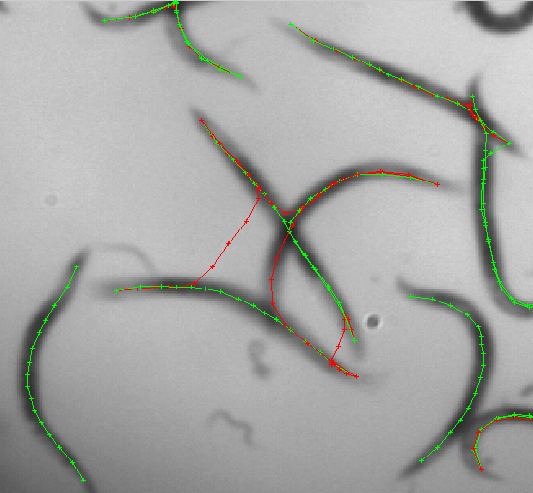
0.479 0.924 0.473 0.352 0.901 0.338

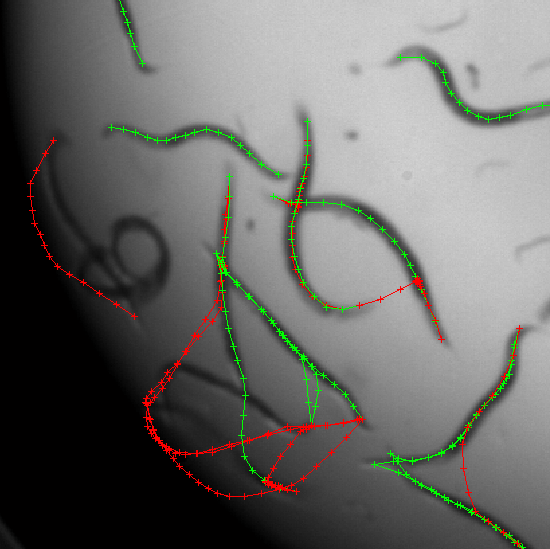
*Len + Sym +Acr*

*E*

0.747 0.924 0.743 0.736 0.923 0.732

*c ont ou r*





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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 co mparable with non-energy based features that

despite of detecting most nematode contours have a high rate of false detections. The

total contour energy

*E*

performed poorly.

*c ont our*

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*

*,*

*S*

and

*e*

*e*

*M*

*+*

*S*

features is the tension energy term

*e*

, the spatial distrib ution of contro l points

*e*

*e*

*t*

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 nematod es show

lower external energy

*e*

values near the center, control points

*e x t*

tend to gather in that area however as they move

*e*

increases in the vicinity of contour

*t*

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

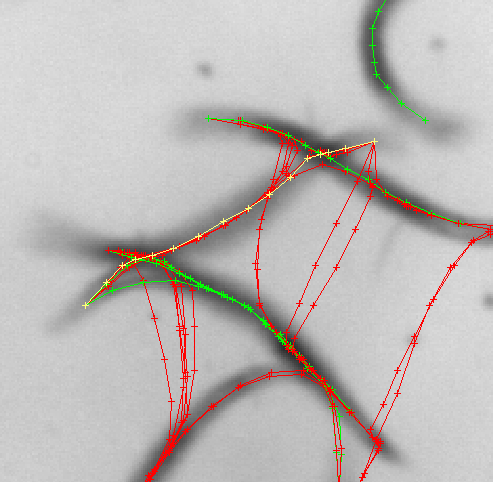
combining several energy terms the false positive rate can be consistently reduced. As

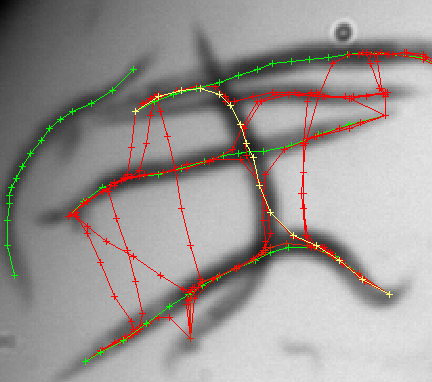
expected bending energy

*e*

allow us to filter out contours with sharp turns and the

*b*





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external energy

*e*

, those with spatial intensity distribution too different from those

*e x t*

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 relatio n 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 keep ing a

significant number of correct detected nematodes.

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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 sho w 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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