Categorizing cells in phytoplankton images *

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Abstract: This article is concerned with detection of invasive species—Prorocentrum minimum (P. minimum)—in phytoplankton images. The species is known to cause harmful blooms in many estuarine and coastal environments. A new technique, combining phase congruency-based detection of circular objects in images, stochastic optimization, image segmentation, and SVM and random forest-based classification of objects was developed to solve the task. The developed algorithms were tested using 114 images of 1280 × 960 pixels. There were 2088 P. minimum cells in the images in total. The algorithms were able to detect 93.25% of objects representing P. minimum cells and correctly classify 94.9% of all objects. The results are rather encouraging and will be used to develop an automated system for obtaining abundance estimates of the species.

Key–Words: Phase congruency, Detection of circular objects, SVM, Random forests, Stochastic optimization, Phytoplankton

1 Introduction

Studies of long-term changes in aquatic ecosystems, monitoring of toxic algal blooms, assessment of water quality parameters are some examples, where identification and counting of plankton cells is being used. Much work in this area still remains in the form of conventional microscope analysis and is very time consuming and labor intensive. A robust automated image analysis-based system would be of great help and would enable analysis at much larger scales. However, developments in the field of automated analysis of phytoplankton images are rather limited. Work by Gorsky et al. [1] is one of the pioneering attempts in this area [1]. Using simple geometric features the authors were able to distinguish between 3 species of distinct size and shape. Blaschko et al. [2] achieved 50% to 70% classification accuracy in a task of phytoplankton categorization into 12 classes plus an “unknown” class. A large variety of features: shape features, moments, texture features, contour features (780 features in total) were used. Several classifiers, including decision trees, naive Bayes, ridge linear regression, k-NN, SVM, and bagged as well as boosted ensembles were explored. SVM was found to be the best classifier for the task. Culverhouse et al. [3] studied the classification accuracy achieved by the neural network committee-based automated system DiCANN and argued that accuracy of about 72% achieved by the system in a six-class phytoplankton categorization task was similar to the accuracy achieved by the trained personnel. Sosik and Olson [4] presented, perhaps, the most elaborate study regarding multi-class phytoplankton categorization using data obtained from Imaging FlowCytobot [5]. In total, 6600 manually inspected images distributed across 22 categories were used. The overall accuracy of 88% was achieved on the test set. While the obtained accuracy is very encouraging, one very important task—object detection—is not addressed in the article.

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Analysis of the literature shows that classification accuracy achieved when solving phytoplankton classification problems varies in a broad range depending on the task and the data. It is worth noting that one very important problem, namely object detection, is almost never addressed in the literature. Robust object detection, however, is a prerequisite for obtaining a robust system for automated analysis of plankton images, especially when rather simple imaging systems, generating images exemplified in Fig. 1 (there are overlapping objects and/or objects touching each other in the images), are used. Touching organisms bring difficulties in automated categorization and counting of objects. A linear relation between the number of items and the automatic counting holds if the percentage of image area occupied by the items remains below 3%. Above this threshold, automatic counting underestimates abundance due to the increased percentage of organisms touching each other [6].

In contrast to previous techniques, a simple imaging system is used to obtain images in this study. A long term goal of this work is an automated system for image analysis- and soft computing-based detection, recognition and counting of objects representing different phytoplankton species. This article is limited, however, to analysis of one invasive species, a Prorocentrum minimum (P. minimum), which is known to cause harmful blooms in many estuarine and coastal environments [7]. A new technique, combining phase congruency-based detection of circular objects, stochastic optimization, image segmentation, and SVM and Random forests-based classification was developed for solving the task.

2 Data
Phytoplankton samples used for obtaining images were received from: 1) natural south-eastern Baltic Sea phytoplankton containing P. minimum cells, 2) cultured P. minimum; and, 3) natural phytoplankton mixed with cultured P. minimum. All samples were fixed with acetic Lugols solution (in proportion: 0.5 ml of solution for 100 ml of sample). Circular and oval are the dominant shapes of P. minimum cells. The length of P. minimum varied from 14 to 22 µm, while the width ranged from 12 to 18 µm.

Images for the analysis were obtained from a color camera of 1280 × 960 pixels attached to an inverted microscope with magnification of 400x. Fig. 1 presents an image example, where three decision classes are identified, namely the invasive (an example of this class is enclosed in a rectangle), native (inclosed in a circle), and other (enclosed in a pentagon). Invasive (P. minimum) cells dominate the image. In total, 114 images have been collected for the analysis at several occasions.

3 Methods
3.1 Image preprocessing
A clear 2D shell is a characteristic feature of the invasive cells. To emphasize the feature, we applied preprocessing via the phase congruency-based enhancement of image edges [8]. The phase congruency idea is based on the assumption that features (edges, corners) are perceived in image points, where the signal Fourier components are maximally in phase. The task of Fourier analysis applied here is to give local frequency information. As suggested by Kovesi [8], we obtain this information by applying a bank of Gabor filters tuned to different spatial frequencies rather than via Fourier analysis. When working with 2D images, local energy is first computed in several orientations θ using 2D Gabor filters [8] and 2D phase congruency PC(x) is then computed according to the following equations:

$$PC(x) = \frac{\sum_\theta \sum_n w_\theta(x) A_{\theta,n}(x) \Delta \Phi_{\theta,n}(x) - T_\theta}{\sum_\theta \sum_n A_{\theta,n}(x) + \varepsilon}$$ (1)

$$\Delta \Phi(x) = \cos(\phi_n(x) - \bar{\phi}(x)) - |\sin(\phi_n(x) - \bar{\phi}(x))|$$ (2)

where the index θ runs over orientations, A_{\theta,n}(x) and \phi_n(x) is an amplitude and a phase angle, respectively, of the nth component at the location x, \bar{\phi}(x) is the amplitude weighted mean phase angle, w(x) is a parameter weighting frequency spread, and ε is a small constant, T is a parameter meaning that only energy values exceeding T influence PC, and \lfloor y \rfloor = y if y > 0 and \lfloor y \rfloor = 0 otherwise.
We use a PC image to extract objects in an original phytoplankton image. We also utilize information of phase congruency variation with orientation. This information is obtained from an angle image $\Phi$ (an angle of the principal axis about which the phase congruency moment is minimized [9]) and an image of the magnitude of the maximum moment $M$ (the moment about an axis perpendicular to the principal axis [9]) and the minimum moment $m$ of phase congruency. Values of $\Phi$, $M$, and $m$ computed in each image pixel, are given by [9]:

$$\Phi = \frac{1}{2}\tan^{-1}\left(\frac{b}{\sqrt{b^2+(a-c)^2}} - \frac{a-c}{\sqrt{b^2+(a-c)^2}}\right)$$  \hspace{1cm} (3)$$

$$M = \frac{1}{2}\left[c + a + \sqrt{b^2 + (a-c)^2}\right]$$  \hspace{1cm} (4)$$

$$m = \frac{1}{2}\left[c + a - \sqrt{b^2 + (a-c)^2}\right]$$  \hspace{1cm} (5)$$

where

$$a = \sum_{\theta}[PC(\theta)\cos(\theta)]^2$$  \hspace{1cm} (6)$$

$$b = 2\sum_{\theta}[PC(\theta)\cos(\theta)][PC(\theta)\sin(\theta)]$$  \hspace{1cm} (7)$$

$$c = \sum_{\theta}[PC(\theta)\sin(\theta)]^2$$  \hspace{1cm} (8)$$

where $PC(\theta)$ is the phase congruency value computed at $\theta$ using Eq. (1).

### 3.2 Determining centre points of circular-shaped objects

$P.\ min\ imum$ cells are approximately circular- or oval-shaped, see Fig. 1. To facilitate the cell detection step, a technique for determining centre points of circular objects was developed, based on $M$ and $\Phi$ images. One can consider image $M$ as an image of edge clarity (certainty) values in each pixel of the original image, while image $\Phi$ reflects edge direction in each image pixel. Fig. 2 presents an example of an original image and corresponding $M$ and $\Phi$ images. Image $C$ reflecting clarity (certainty) of a centre point in each image point is created first. The size of $C$ is equal to the size of the original image, $m \times n$. We start by setting $C_{ij} = 0, \forall i, j$. Image $C$ is then obtained by applying the following algorithm, where $r$ is the supposed radius of an object.

For $i = 1, ..., m$

For $j = 1, ..., n$

$$i^+ = i + r \sin(\Phi_{ij}+90), \hspace{1cm} j^+ = j + r \cos(\Phi_{ij}+90)$$

$$i^- = i - r \sin(\Phi_{ij}+90), \hspace{1cm} j^- = j - r \cos(\Phi_{ij}+90)$$

if $0 < i^+ \leq m$ and $0 < j^- \leq n$

$$C_{i^+,j^+} = C_{i^+,j^+} + M_{ij}$$

end (if)

The idea is similar to that used in the Hough transform. However, our technique is much more robust to noise. Since shape of $P.\ min\ imum$ cells deviates from a circle, many pixels in the vicinity of the centre position exhibit high $C_{ij}$ values. Moreover, $P.\ min\ imum$ cells differ in size. Therefore, $C$ images are filtered by a rotationally symmetric Gaussian low-pass filter with $\sigma = 3$.

### 3.3 Determining contour of circular objects

Having a centre position of a circular-shaped object, a square of size $(2r + \beta) \times (2r + \beta)$, where $\beta$ is a parameter, is clipped out around the centre together with corresponding $M$ image for further analysis. To emphasize image elements making horizontal lines and to suppress isolated image elements, $M$ image, transformed to a polar coordinate system, is filtered by a Gaussian filter with a much larger standard deviation in the angle direction than in the $r$ direction.

An iterative algorithm is then used to find a contour line $c$. The algorithm aims at placing a contour line in high intensity points of a filtered $M$ image $E$. For each $\alpha$, the initial contour position $c_0^{\alpha}, \alpha = 0, ..., 360^\circ$, is given by the largest $r$ value, such that $E_{\alpha,r} > \text{mean}(E_{\alpha,r})$:

$$c_0^{\alpha} = \max (\text{arg}_r [E_{\alpha,r} > \text{mean}(E_{\alpha,r})])$$  \hspace{1cm} (9)$$

where $E_{\alpha,r}$ is an image $E$ element – intensity at angle...
\( \alpha \) and radius \( r \). At each iteration, the contour line \( c \) is updated by \( \Delta c \):

\[
e^{i+1} = e^i + \Delta c, \quad \Delta c = B_{\text{step}} \ast G
\]

where \( i \) denotes an iteration index, \( B_{\text{step}} \) stands for a vector of best step sizes at different \( \alpha \) and the convolution operation, denoted by \( \ast \), with a Gaussian filter \( G \) is applied aiming the adjustments at different \( \alpha \) to be dependent on neighbours. At \( i \)th iteration, the best step size \( B_{\text{step}} \alpha r \) at the randomly selected angle \( \alpha r \) and using the Gaussian window of randomly selected width \( w^r \) is given by:

\[
B_{\text{step}} \alpha r = \arg \max_{\text{step}} \sum_{j=-w^r}^{j=w^r} E_{j, c_{j} + \text{step}} G(j)
\]

where \( G \) is a Gaussian window with maximum at \( G(0) \) and \( j^r = \alpha r + j \). If \( B_{\text{step}} \alpha r \neq 0 \), contour adaptation takes place at \( \alpha r \) and in the neighbourhood of \( \alpha r \), given by \( \alpha r \pm w^r \):

\[
e^{j+1} = c_{j} + B_{\text{step}} \alpha r \cdot G(j^r - \alpha r), \quad j^r = \alpha r - w^r, ... \alpha r + w^r
\]

The width of the Gaussian window \( w^r \) is randomly selected from the interval \( w_{\text{min}} = 20 \) and \( w_{\text{max}} = 100 \). The search continues for \( n_s \) successful iterations, \( B_{\text{step}} \alpha r \neq 0 \), or for \( n_o \) iterations in total. Values of \( n_s = 40 \) and \( n_o = 1000 \), found experimentally, worked well in all the tests.

### 3.4 Segmenting original images

To detect objects, not only phase congruency preprocessed images, but also original colour images were used. Original RGB images were first transformed to the Lab colour space and then used in the segmentation process, based on the Fuzzy C-Means clustering (FCM) algorithm. In fact, segmentation into two clusters, "objects" and "background" is needed in this study. However, aiming to obtain more accurate object boundaries, a larger number of clusters was used in the FCM algorithm. Then, in the next step, clusters were merged and too large regions, representing the background, were eliminated.

### 3.5 Combining analysis results for object detection

We do not consider very small and very large objects, and objects with centre points too close to image boundaries. It is well known that fusing results obtained from different analysis techniques is an efficient way to improve the accuracy of the analysis [10, 11]. Therefore, results obtained from the different techniques were combined. The algorithm can be summarized by the following steps:

i. create an image of objects \( O \) by segmenting an Lab image;
ii. determine centres of circular objects;
iii. determine contour lines of the circular objects;
iv. eliminate pixels belonging to the circular objects from \( O \); this step ends the phase I of the algorithm.

v. eliminate pixels belonging to very small and very large objects from \( O \); objects much smaller than \( P_{\text{minimum}} \) cells are assumed to be very small;
vi. if the image \( O \) contains at least one object, take an object from \( O \) and perform the following steps (phase II):

(a) determine a centre point of the object; if the object is larger than twice the average \( P_{\text{minimum}} \) cell size, the most distant point from the object edge is assumed to be the centre point;
(b) determine a contour line of the object and eliminate its pixels from \( O \);
(c) apply the morphological opening operation on the image \( O \);
(d) eliminate very small objects from \( O \);
(e) if \( O \) contains at least one object go to Step (vi); otherwise stop.

### 4 Feature extraction

In total, 65 features characterizing object geometry and texture were used. The feature list includes: (1) object area; (2) eccentricity; (3) perimeter; (4-10) the Hu set of invariant moments (7 moments) [12]; (11) standard deviation of object grey levels; (12) entropy of the object grey levels; (13-14) mean and standard deviation of the local entropy of object grey levels (the entropy value in the \( 9 \times 9 \) neighbourhood of each pixel of the input grey scale image is computed); (15-16) mean and standard deviation of the local standard deviation (the \( 3 \times 3 \) window is used to compute the local standard deviation); (17-30) fourteen Haralick’s [13] coefficients computed from the averaged (over four directions) co-occurrence matrices estimated using the distance parameter \( d = 5 \); (31-34) four features representing the mean intensity and (35-38) four features representing the standard deviation of intensity of the input image filtered using Gabor filters of four different scales: 2.5, 5, 10, and 20 (six orientations \( 30^o, 60^o, 90^o, 120^o, 150^o, \) and \( 180^o \) are used and the results are averaged); (39-40) mean and standard deviation of \( M \) image pixels corresponding to the object; (41-42) mean and standard deviation of \( C \) image pixels corresponding to the object; (43-44) mean and standard deviation of \( C \) image pixels corresponding to the object, (45) mean of binary object image obtained from the FCM clustering; (46-55) ten
features computed from the local standard deviation image to characterize the variation of average intensity values when going from object exterior towards the object center (30 steps are used), where the average intensity is represented by the average intensity of points on the shrinking object perimeter line; in this way a vector \( z \) of 30 average intensity values is obtained and the following features are then computed: (46-47) mean and standard deviation of the average intensities; (48-55) the first eight coefficients of the Discrete Cosine Transform of \( z \); (56-65) computed in the same way as (46-55), except that \( M \) image is used instead of the local standard deviation image.

5 Classification

A committee of SVM and random forest (RF) [14, 15] is used to make a decision. Aggregation of SVM and RF is done by averaging a posteriori probabilities obtained from these two classifiers. The a posteriori probability from a trained RF is estimated as:

\[
p(t_1, \ldots, t_L, r, q) = \frac{\sum_{i=1}^{L} f(t_i, r, q)}{L}
\]

where \( t_1, \ldots, t_L \) are trees of the random forest, \( r \) is the object being classified, \( q \) is a class label, and \( f(t_i, r, q) \) stands for the \( q \)th class frequency in the leaf node where \( r \) falls in the \( i \)th tree \( t_i \) of the forest:

\[
f(t_i, r, q) = \frac{n(t_i, r, q)}{\sum_{j=1}^{Q} n(t_i, r, q_j)}
\]

where \( n(t_i, r, q) \) is the number of training data coming from class \( q \) and falling into the same leaf node of \( t_i \) as \( r \).

The optimal values of SVM hyper-parameters (width of the Gaussian kernel and the regularization constant) and the optimal feature set for the classifier are selected by genetic search [16]. The optimal feature set for RF is found by sequential backward elimination.

6 Experimental investigations

Using the a priori knowledge about the size of \( P. minimum \) cells, the supposed radius of an object \( r \) was set to \( r = 45 \). An \( M \) image in the polar coordinate system is filtered by a Gaussian filter with a much larger standard deviation in the angle direction than in the \( r \) direction. The standard deviation equal to 8 and 1 in the angle and \( r \) directions, respectively, was a good choice. The width of the Gaussian window \( w^r \) used by the stochastic contour correction algorithm is randomly selected from the interval \( w_{\text{min}} = 20 \) and \( w_{\text{max}} = 100 \). The number of successful iterations

\[
n_s \quad \text{and the total number of iterations} \quad n_o \quad \text{were set to} \quad n_s = 40 \quad \text{and} \quad n_o = 1000.
\]

Objects larger than five times the average cell size are considered as being very large. The optimal number of randomly selected features used to split a tree node in RF was equal to 13. It is worth mentioning that the algorithms are rather insensitive to the choice of the parameters.

6.1 Results

Fig. 3 (top) presents an example of object detection results for a full-size image. As can be seen from the figure, majority of small "uninteresting" objects were eliminated and all \( P. minimum \) cells, except those with centre points too close to the image boundaries, were detected. All objects detected in phase II do not belong to the class of \( P. minimum \) cells. Fig. 3 (bottom) presents one more example of object detection results. Objects marked by yellow crosses were left aside from the analysis, as being too close to the image boundaries if compared to the chosen parameter \( r \) value.

In total, 114 images were processed and the detection results were verified by manual inspection. The manual inspection has shown that there are 2088 \( P. minimum \) cells in these images in total. The algorithms found 2412 objects. Among these objects, 1947 were \( P. minimum \) cells. Thus, 93.2% of \( P. minimum \) cells were detected. The test data set classification accuracy obtained from the SVM, RF, and the committee was equal to 94.4%, 90.9%, and 94.9%, respectively. The accuracy presented here is the average.
accuracy obtained from 20 trails using different random split of the data set into learning and test subsets.

7 Discussion and Conclusions

An automated system for detection, recognition, and derivation of abundance estimates of different phytoplankton species using ordinary phytoplankton images, generated by a simple imaging system, is a long term goal of this work. This article was concerned with detection and categorization of objects representing one invasive species, a *P. minimum*, which is known to cause harmful blooms in many estuarine and coastal environments. Special emphasis is made on the object detection problem. Nonetheless robust object detection is a prerequisite for obtaining a robust system for automated analysis of plankton images, the problem is almost never addressed in the literature.

A new technique, combining phase congruency-based detection of circular objects, stochastic optimization, and image segmentation was developed for solving the object detection task. A committee consisting of a support vector machine and a random forest is used to make a decision. A committee decision is obtained by averaging the a posteriori class probabilities obtained from these two classifiers. Experimental tests have shown that robust object detection is possible even in images, where image area occupied by objects was much larger than 3%. On average 93.25% of objects representing *P. minimum* cells were detected. The test data set classification accuracy of 94.9% was obtained from the committee. It is worth mentioning that even objects extracted from rather unfocussed images were used in the experiments. Bearing in mind the simplicity of the imaging system used and high percentage of image area occupied by objects in phytoplankton images, the obtained results are rather encouraging.

References:


