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Automatic Detection and Identification of Morphological Growth Patterns of Chroococcus Minutus

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***Abstract* - The aim of the present study is to develop an automatic identification tool which is used to detect and classify the given Cyano bacterial digital cell images morphological stages. The different geometrical features are considered to detect, identify and classify the different morphological stages of blue- green algal cell such as about-to divide, normal and grownup. We propose a computerized method for segmentation and classification based on active contours and rule based classifier for the morphological phases of the Cyano bacterial images, and extracting the geometrical features associated with the segmented Cyano bacterial cell images. The experimental results that are obtained from this automated system are compared with the manual results obtained by human experts in the field of microbiology and discussed the efficiency and accuracy of the proposed system.**

I. INTRODUCTION

These Algae are found in bulk and they are highly sensitive to the changes to their environment. The survey results carried out in various lakes in different countries worldwide has stated that 75 of the various lake water sample contains toxic blue-green genera. This led many researchers to develop automated systems to identify and classify the blue-green algae. several studies were carried out in India on biodiversity of algae [1], [2], [3], [4] Over the last few decades, the various traditional methods that were used to recognize, detect and classify each of the individual algae, whether in a given water, soil sample, preparation of algae slide sample under a microscope or identification of algae genus with help of human expert. All the above stated methods are time consuming, tedious and subject to human error. Recent computer based image processing techniques in combination with other approaches are used to develop an automated system to identify and classify the microbial world. The comparative study between the manual and the automated processes of recognition is based on these factors like speed, accuracy, reliability and cost. Cyan bacteria are a group of gram negative prokaryotes. The diversity of cyan bacteria ranges from unicellular to multicellular, branched to coccoid filaments,autotropic to heterotrophic [3]. These organisms are found both free living and as endosymbionts. Cyan bacteria are found in bulk and they are highly sensitive to the changes to their environment. Cyan bacteria share a very close resemblance with the eukaryotic algae, having similar habitats and often grow together. The survey results carried out in various lakes in different countries world wide has stated that 75 of the various lake water sample contains toxic blue green genera. This led many researchers to develop automated systems to identify and classify the blue-green algae [18]. Chroococcus are uncials that are ovoid or rod-shaped with a diameter which ranges between 0:4to40m [?]. Chroococcus inhabit in freshwater areas and also in water sources of higher salinity.

A. Related work

Few group of methods were designed earlier for detection and identification of diatom which was proposed by Cairns [17]. Later Culverhouse in 1996 derived methods based on neural networks for identification for phytoplankton. later, Alvarez Borrego in 1998 have proposed a hybrid optical digital method for detection and identitification. In 1999 another technique called correlation were used for identification. Based on histogram thresholding and computation of variance of the image gradient, modular localization were few other methods used in identification. These are one of the potential organisms which are useful for the mankind in many ways. Cyano bacteria, they are also referred as blue-green algae, and they are responsible for creation of oxygenic photosynthesis. These organisms has drawn the world wide attention for their possible use in the field of food, fuel, fertilizers, colourant, mariculture, feed [?] and also used in the various secondary metabolites including toxin [?], enzymes, vitamins etc. Few Cyano bacteria

causes algal blooms in the fresh water [15]. An image analysis algorithm was constructed to measure and isolate the objects from the phytoplankton image using the combination of artificial neural network and rule based classifier. Water samples from various lakes of Japan were collected and an automation mechanism was generated in detecting and recognition of four genera of Cyano bacteria using artificial neural network. In the previous study they made use Sequential Minimal Optimization as classifier to classify the algae [5].

B. Features of algae under study

We report here the features that distinguish the species of algae considered in this study. Choococcales contains two families one by named hoococcaceae and the other Entophysalidaceae.Chroococcoccus are single celled or after division as a tetrad of cells within a common sheath ranges from 3 - 10 microns. The species Choococcales has two genera that are significant to our study : Chroococcus Minitus and Chroococcus Minor. Chroococcus Minitus ,the members are single celled or occurs in group of 24 cells under a common sheath, which are spherical or oblong in shape , colorless and not lamellate as shown in the below fig. Automating the analysis of microbial images has made the study of microbial ecology more efficient. Computer based techniques are used to enhance the images , segment the region of interest and quantify cell volume growth rate.

II. RESEARCH OBJECTIVES

Over the last few decades, the various traditional methods that were used to recognize, detect and classify each of the individual algae, whether in a given water, soil sample, preparation of algae slide sample under a microscope or identification of algae genus with help of human expert. All the above stated methods are time consuming, tedious and subject to human error. Recent computer based image processing techniques in combination with other approaches are used to develop an automated system to identify and classify the microbial world. The comparative study between the manual and the automated processes of recognition is based on these factors like speed, accuracy, reliability and cost.

III. MATERIALS AND METHODS

The imaging for this study was performed in Department of Microbiology Bharati Dasan University Tiruchinapalli, India.They all were enriched in BG-11 medium .The cultures were incubated in growth room maintained at 27 degree, 1500 lux and 16 hour light and 8 hour dark cycle for 15 days for BG 11 to get the optimum growth of the organism (Blue-Green algae).Then the different growth patterns of the cells were captured through the bright field microscope attached with Tuesen USB 2:0H series camera. Over 300 cells of each algal genera were collected .The data set was partitioned into mutually exclusive training and testing cells.

IV. PROPOSED SYSTEM

The proposed system has four components as shown in fig 1. Preprocessing Feature Segmentation Classification Extraction Fig. 1. A schematic block diagram of the proposed system The advantages of making use of computerized system for automation can be stated as :Automatic recognition, classification of an organism is much faster than the traditional ways Computer based calculation are more accurate than the accuracy obtained in the conventional method as these methods are subjected to expert knowledge .The cost involved in identification and classification of the algae manually is more costlier than the automated one. Learning and training of the data in automated is much cheaper than employing the human power. Automated helps us monitoring the data online data monitoring where as the traditional way does not support for the real time online data monitoring.

A Pre-processing

The captured digital cell images usually suffer from different problems such as darkness, color,unwanted objects, noise. Pre-processing is performed on the data set to render digital image amenable for further processing. Since both species appear the same(in terms of color) under bright field microscopy, as a first step, we convert the RGB image to grayscale. This gray scale image is not uniformly illuminated. Further, the range of gray scale values is highly limited. Consequently, there are large portions of dark regions that may obscure details from the segmentation algorithm. To overcome this, we perform histogram equalization, to enhance the contrast and ensure that all cells are visible to the segmentation process.

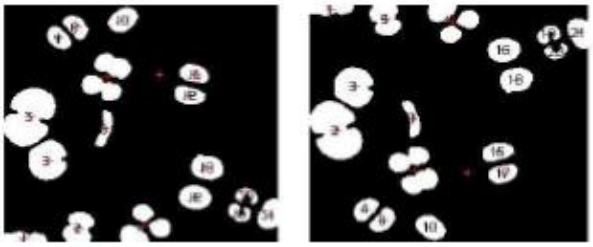
B. Segmentation

In this paper, we propose a system to segment cells in algal images and then classify the species based on the features extracted from the segmented cells. Further, we also find the growth stages (single celled, dividing or adult)based on the number of cells present in the common sheath for both Chroococcus Minitus and Chroococcus Minor. Hand segmentation of these cells is tedious hence, automation is a robust and time-efficient to quantify the growth rate of these cells. Various edge detection techniques are used to extract edges. The edge detection techniques when implemented on our training set cell images the results are not satisfactory as shown in the below fig. The most extensively used method in image segmentation and object detection is active contour also

called as snake model. In this method the segmentation is done using the deformable curves. This method consists of creating initial contour which is placed near the object of interest in the given cell image, then the contour is effected by internal and external energy. The internal energy helps to maintain the smoothing of the active contour and the external energy will help the active contour and the active contour to move towards the borders of the desired objects in a given image. In our study we segment the given cell images using active contours which give better segmentation results as shown in the fig. The snake which we used for segmentation the initial mask is adjusted and the numbers of iterations are taken care so that we get the accurate segmented images.

C. Feature Extraction

This process of extracting the suitable parameter and the different values of the digital images that are used for the recognition and identification of the images of the species. Feature extraction makes use of the geometrical parameters such as area, perimeter, and centroid. Area defines the size of the given object in an image. Centroid helps use to know the center coordinates of the object .Further, automatic labeling for the image is done refer fig. labeled Image Centroid image



D. Classification

Classification is an important field in data mining for a classifying a given problem . Given a set of labeled training images as examples the classification task constructs a classifier. Numerous classification techniques are available in literature, among them we are making use of rule-based classifier. In our paper we propose rule based classifier which makes use of collection of IF-THEN statements for classification. The general format is as follows: IF condition THEN conclusion. The IF part in the condition is called as the precondition and the THEN part is called as consequent. To build a rule based classifier, we need to extract a set of rules that show the relationships between the attributes of a data set (digital cell images) and the class label. Here, The rule based classifier is used for classifying the segmented images making use of geometrical features like area, perimeter etc. based on the results the cells are classified as single celled, dividing or adult based on the number of cells present within the common sheath.

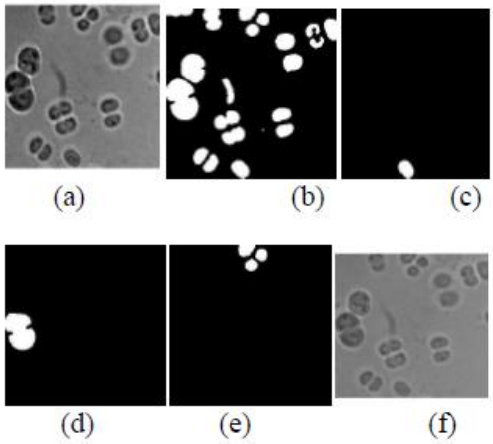
a.one cell b.two cell c.three cell d.four cell

Algorithm 1 Training phase steps
Step1: Input the captured microscopic image to the system .
Step 2: convert the RGB image into gray scale images.
Step 3: Implement prepossessing method if required.
Step 4: Segment the resulting gray images using active contours.
Step 5: After segmenting the digital image Perform labeling of the image.
Step 6: For each labeled segment, compute. area, perimeter, centroid,eccentricity, roundness for each cell phase k to distinguish between about-to- divide
Step 7: Repeat the steps from 1 to 5 for all the blue green algal images.

Algorithm 2 Classification phase steps
1: Input the captured microscopic image to the system .
2: convert the RGB image into gray scale images.
3: Implement prepossessing method if required.
4: Segment the resulting gray images using active contours.
5: After segmenting the digital image perform labeling of the image.
6: For each labeled segment, compute area, perimeter, centroid,eccentricity, roundness for each cell phase k to distinguish between about-to-divide,normal,grownup.
7: Apply rule based classifier for the algal cells to classify it into normal, about-to-grow, grownup.

V. EXPERIMENTAL RESULTS

For experimentation purpose 300 digital Chroococcus Minitus cell images containing different growth patterns cells namely initial stage, about-to divide and adult phases are considered as shown in fig. In training phase, each input digital chroococcus minitus cell images are converted to gray scale image as in fig b and then the cells are preprocessed. Further, on these cells active contours are used to segment the grayscale as in fig c. The segmented image of chroococcus minitus is then labeled and for each labeled region digital cell, the geometric features are calculated. The table 1 summaries the values of the geometric features that are computed from the labeled segmented chroococcus minitus cell regions of the image.



Original color image, (b) gray image of color image in (a), (c) segmented image, (d) the segmented region known as initial, (e) the segmented region known as about-to-divide, (f) the segmented region known as about-to-divide phase, (g) the segmented region known as adult phase

Table1: Geometrical Features

Parameters	Single Celled	Double Celled	Three Celled	Four Celled
Area	573	674	914	1240
Centroid	176.11-47.83	68.9-15.01	129.43-17.17	95.02-174.4
Perimeter	88.18	109.01	174.62	114.32
EquivD	27.01	29.29	33.11	39.73
Roundness	0.9250	0.7125	2.0621	1.192

VI. CONCLUSION

In the present study we propose an automated system to find the various growth patterns of Chroococcus minitus by segmenting the digital microscopic images and classification based on geometrical features of the cells. The experimental study of the proposed system is compared with the manual results obtained by the phycologist. This system is less expensive and yields better results.

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