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Automatic Detection and Pattern recognition of Morphological growth phases of *Scenedesmus sp*

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Abstract: *This study presents an automated system for identifying and classifying the growth patterns of Scenedesmus sp, a type of cyanobacteria. Different geometrical features associated with the species are used to detect, identify and classify cell growth patterns of the genera. The workflow proposed for segmentation comprises active contours and mathematical morphological operators and classification comprises a rule- based classifier or decision trees to delineate cell growth patterns as different morphological phases. The methods proposed have been compared against the gold standard – images segmented and labeled by human experts – and the results are found to be promising.*

Keywords: *Cyanobacteria, Morphological operations, classification, active contours, senedesmus sp.*

I. INTRODUCTION

Nowadays, there are many more commercial applications of microalgae that are used to enhance the nutritional value used in the food and the animal feed depending on the chemical composition [1,2]. These organisms also play an important role in the aquaculture and also they are used in best possible use in cosmetics, poultry, and pharmaceutical because of the presence of different useful entities. Microalgae serve a food colorant, high-value molecules for human food. Microalgae are also employed in the field of agriculture as bio fertilizers and as soil conditioners and also helps in maintaining and building up the soil fertility. Microalgae feeds are used currently as the culture of larvae and juvenile shell and finfish. Despite these advantages, there are some problems posed by algae. These organisms affect the water quality by affecting properties of water such as taste, odors and chemical properties which cause hazards to human beings and animals [3]. Some algae produce toxins that harm the water quality and aquatic animals [4-9]. Nearly 47% of Scenedesmus quadricanda is protein[10]. Even though Scenedesmus is one of the most common freshwater genera of macroalgae, the identification of this species is difficult due to diverse morphologies found within the species. Scenedesmus sp. exist as unicells or these species also found in coenobia of four or eight cells within a parental mother wall [11]. These species exhibit different coenobia architectural patterns such as linear, irregular, costulatoid [12]. Studies on Scenedesmus have proved that they are efficient at removing ammonia in the cylindrical bioreactors and have been reported to present large amounts of long-chain hydrocarbons. *Scenedesmus sp* is used in removing the cadmium cation from aqueous solutions. Two more species of Scenedesmus quadricanda are used in the production of biomass and co2 mitigation through photosynthesis to produce the biomass [12]. Scenedesmus strain R-16 which is used in lipid production [13,14]. Petroleum coal and natural gas conventional fossil fuels play a dominant role in the global energy consumption [15]. All these traditional fuels are nonrenewable with limited reserves and in turn increasing cost [16]. The next promising method which is economically feasible

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and can meet the growing demand and acts as an alternative fuel for energy. Traditionally the biodiesel contains plant oils and animals fats [17] which are very hard to satisfy all long-term global energy demand. In order to do this microalga is widely considered as a potential and efficient way for biodiesel production technology [18, 19]. The utility of this genera motivates the automation of detection and identification for the present study . There have been efforts in the past to automate the detection, classification, etc., of algal images. [22,23]. Such automation has reduced the burden of taxonomists [24]. It also serves as an aid for budding phycologists, supporting the identification and patterning of algal cells – an advantage we hope to harness through the automated system proposed here. Online monitoring of water bodies is useful to detect harmful algae and track the growth of useful species. Since automation of the segmentation, identification, and tracking of cells is prevalent in medical imaging and other fields of biology (such as proteomics, microbiology, etc.), online tools have been developed to carry out basic operations such as enhancing images, segmentation based on edge detection or active contours and morphological operators, followed by the use of generic classifiers such as artificial neural networks for the classification of patterns [25,26]. We propose here a framework that detects the presence of algal cells in a digital image, segments the cell and identifies the species and growth stage (based on the number of cells within the sheath), for which we have not found any publicly available data base or results in the literature, thus making it a useful first effort in the field of phycology.

II. MATERIALS AND METHODS

Cyanobacteria were chosen from the culture bank of department of Microbiology, Bharathidasan University, Tiruchirapalli, grown in BG -11 culture.

2.1 Image processing techniques

Automated processing of images involves various steps, namely preprocessing, segmentation, feature extraction, morphological operations, classification, and identification. The Architectural layout of the image processing method used for detection and classification of algae is as shown below Fig1. Fig. 1 shows a generic system for training a classifier to identify patterns within an image based on a dataset of annotated images and storing the patterns for automated classification of patterns in a new image. The image processing techniques involved are discussed in detail in the following section.

2.2 Image preprocessing

The images of the object are captured using a microscope attached to a camera and these digital images if they contain noise then with the help of the median filters the noise present in the image is removed.The Median filter is a digital nonlinear filtering technique that is often used to remove some kind of noise reduction on an image or in some signal. This is a pre-processing step to get improvement in the results for further processing i.e for segmentation using edge detection. The main idea of median filters is to replace each entry with the median of the neighboring entries[27,28]. It is the most widely used filtering techniques as it prevents edges and removes noise under certain conditions. It is one kind of smoothing technique as shown in figure 2. And Image enhancement Is an important process to remove the unwanted distortion that is caused due to deterioration in the contrast. Improper distribution of the intensity, unwanted noise etc. The contrast of an image is defined as it is image element that is defined as the ratio between the highest and the lowest intensities of pixels that are present in an image under consideration. Thus, Histogram equalization method produces the output image with a uniform distribution of pixel intensity. Histogram equalization method produces the output image with uniform distributions of pixel intensity. It is one of the effective contrast enhancement techniques. The steps that are followed for HE are: Input an image, get the histogram of the image, find the local minima in the histogram. Based on this image histogram is divided and on each partition of the histogram, histogram equalization is applied as shown in figure 2. Mathematical morphology is a tool for investigating the geometric structure of binary or gray scale images where meaningful shape information is derived from irrelevant one. All the morphological operation are nothing but a different set of operations that we have to use depends on the kind of morphological transformation of a given image.

2.3 Image Segmentation

One of the important segment of an image processing is segmentation[29,30]. Segmentation is the process of separating the actual image from the background. The same species of algal shows various shapes during the growth phase. In the previous research papers, the automatic identification and classification of algae are done using the edge detection by making use of **Canny's or Sobel's** edge detectors [31-33].For this present species under consideration first edge detection is used for segmentation. The Sobel and Canny edge detectors are used for segmentation[34], but could not find clear structure as the resulting images contain discontinuities as shown in figure 3. In order to improve the quality of output after edge detection, morphological operators were applied so that the segmented image is clear. After segmentation, the next step is to find the various growth stages of the species under consideration. This can be done by labeling the images and then finding the centroids in the digital image by using region props as depicted in the Fig 3.

2.4 Feature extraction

Since the classification technique is highly dependent on features of the objects in an image, it is essential that we get significant information from the features extracted from the objects under study. The various geometric shapes are used as the measure for classification for example perimeter, area, radius, eccentricity, the circularity of the sample images.

Pattern recognition is one of the important field of machine learning which is an act of inputting the raw data and considering action based on the category of patterns[35,36].The pattern recognition algorithm consists of image segmentation ,extraction of features(feature extraction) and classification[37].The object under consideration i.e the raw image is first segmented by using one of the segmentation technique and then the object of interest features are extracted from the segmented image. Then the classifier that is used for can be trained using the features discussed above as the input data. Entire discussion can be depicted in the form of a flow chart that can be used in algal classification as shown in Fig 4.

2.5 Classification

Classification of the image is perhaps the most vital part of digital image analysis. Image classification is a process which helps us to categorize all the pixels in the digital image into one of the several classes. The categorized data then used to produce a systematic representation of different genera of species under observation. The main motive of classification is to identify and classify based on the geometrical features occurring in an image in terms of the object that covers the features which are actually represented by the ground truth by some expertise person in the field of microbiology. All the classification algorithms are based on the fact that the image under consideration exhibits one or more features and one of these features depicts of the several distinct classes. From the literature survey, it is found that decision trees perform better classification than the other classification algorithms [38-40]. In our present study, the decision tree algorithm is adopted to classify the species images. A Decision tree is one of the inductive classification learning algorithms that help us to build classification tree using the training samples/sets. DT is based on the divide and conquers technique[41].Decision trees follow hierarchical structures in which test values are applied to one or more features attributes that may have one of the two outcomes in form of results at each level in the decision 5tree. According to this to classify an object in the image, we began the search at the root of the tree, perform the test, and decide the appropriate branch to the outcome. This procedure continues until a leaf node is encountered, at that time the object in an image is asserted whether it belongs to the species named by the leaf. Based on the geometrical features listed in table 1 ,the species under study is classified into different growth phases of *scenedermus sp*. The first phase, About-to-divide phase ,second phase and final phase as in figure 5.Recall and Precision are the two parameters that are used as the measure for classification which is referred to as true positive predictive rate and positive predictive value .Always the performance of the system is evaluated (computed)using accuracy or percentage over all the predictions. However, always precision and recall are used for each of the class label analysis and individual performance of the class labels or the average the values are computed to get the overall performance of the system. Apart from computing the results for recall and precision , the confusion matrix is the another analyzer used for analyzing the results as it gives very strong in giving the clues where the classifier has gone wrong.

III. EXPERIMENTAL RESULTS

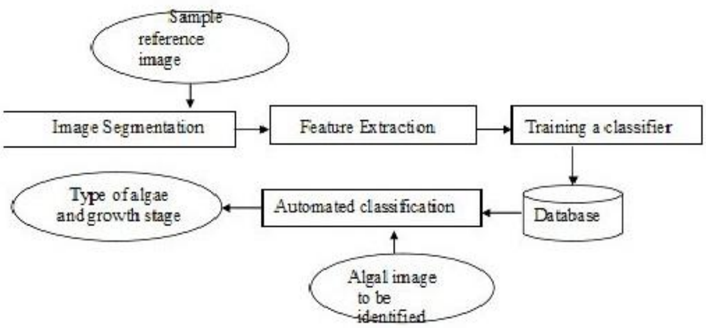

Figure 1a: Steps for algal classification and identification of growth patterns

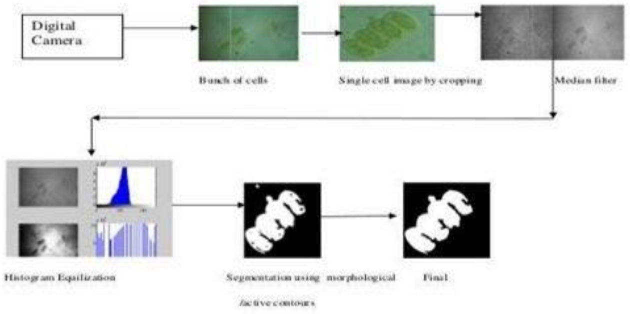

Figure 1b: Architectural Diagram

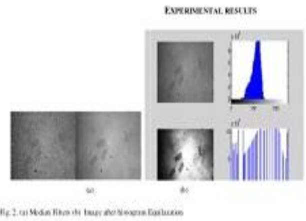

Fig. 2. (a) Median Filters (b) Image after histogram Equalization

Figure 2: a)Median Filters b)Image after histogram Equalization.

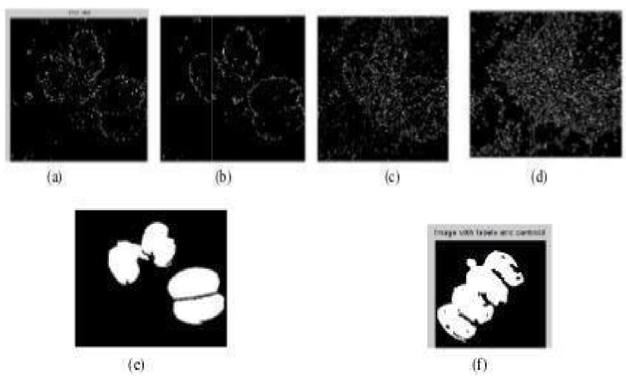

Figure 3: Edge Detection Technique a)Prewitt filter b) Roberts filters c) LoG filters d) Canny filter (e) Segmentation using active contour/ Morphological operators (f) labeled image with the centroid

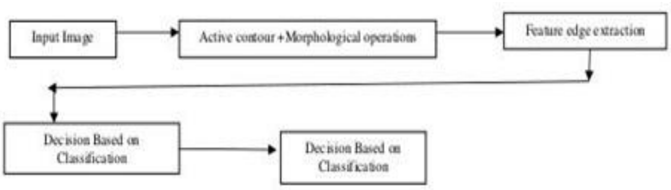

Fig 4:Steps of classification

Figure 4: Steps for Classification

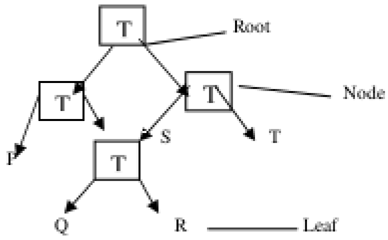

Figure 5: Decision Rule Tree

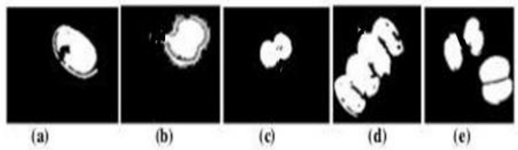

Fig5. Various Growth Phases a)Initial Stage b) About-to-Divide c) Second Stage d) Final Stage e) Multiple growth stages in one slide

Figure 6: Various growth Phases a)Initial Stage b)About-to-divide c) Second stage d) Final Growth Stage e)Multiple growth Stages.

Table 1: Geometrical Features

Geometrical Features	Initial Growth phase	About-to-Divide GrowthPhase	Second Growth Phase	Final Growth Phase
ObjectArea	5516	7960	8264	10755
Centroid	153.3958 to 78.8252	175.1013 to 84.0545	186.1499 to 132.8356	69.44 to 102.52
Perimeter	467.3452	592.0143	615.7128	881.86
Roundness	0.1828	0.4755	0.2854	0.1738
Eccentricity	0.7759	0.6911	0.7006	0.7759

IV. DISCUSSIONS

This work deals with automatically examining the growth phases of cyanobacteria with the help of image processing techniques and pattern recognition. Microscopy is important in microbial ecology it will be more profitable even when used in combination with computer-assisted image analysis in image processing. Experiments are conducted in which first the image is cropped and preprocessed to remove the noise as in fig 2.And then the preprocessed image is then subjected to segmentation is it is depicted in fig 6: These results are obtained with the help of active contours. Where as if we apply edge detection techniques for the same image we are unable to get the clear segmentation as shown in fig 3(a)(b)(c)(d).Then the segmented is labeled and the centroid of the cells in an image are marked as in fig 3(e)and 3(f).Geometric features are extracted and depicted as in table 1.Based on which the given image is classified into initial phase, About-to divide and grown up phases of the genera as shown in fig 6.

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CONCLUSION

In the present study, we exhibit a system which deals with automatically classifying the cyanobacteria genera by automatically identifying the different growth phases of Scenedesmus cyanobacteria. This is possible by taking a microscopic image of cyanobacteria and extract the geometrical feature of this cells in the various phases. The experimental results that are obtained are compared with the manual results gained from the microbiologist who is experts in this area. And the results are much more promising and more over the proposed system is less expensive and less time consuming.

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