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A Novel Approach for Automatic Detection and Classification of Heterocysts from Vegetative cells in *Nostoc* sp.

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Abstract: An automated system is developed by making use of the concepts present of image processing and pattern recognition, for accurate detection, identification and automatically differentiating the vegetative cells and heterocyst in *Nostoc* sp. The motto of this study is to identify the object of interest by using few methods such as edge detection, thresholding to remove unrelated objects from the image. Circular hough transform will detect the object with given radius by collecting maximum voting in a given image.

Key words: Cyanobacteria, heterocysts, *Nostoc* sp., nitogen fixation, circular hough transform, circular hough transform

INTRODUCTION

Cyanobacteria commonly called as Blue-Green Algae (BGA) are found in diverse habitats worldwide (Whitton and Potts, 2000). They are found in freshwater, terrestrial, walls, rocks, stones, tree barks and plant pots and also marine habitats (Boraste *et al*., 2009; Choudhury and Kennedy, 2004; Acea *et al*., 2001). Some of the BGA acts as biofertilizers which can be utilized in improving the plant nutrition effectively, especially the production of rice and wheat (Boraste *et al*., 2009; Choudhury and Kennedy, 2004). Some others commune of cyanobacteria prevents soil erosion helps in soil water retention and also land reclamators (Acea *et al*., 2001). Some of the cyanobacteria are toxic in nature which affects the water bodies farm animals, fish, birds and humans (Zanchett and Oliveira-Filho, 2013; Joehnk *et al*., 2008; Kardinaal *et al*., 2007). In general, cyanobacteria may sometimes consider as a nuisance but they serve as great ecological and agricultural significance in our life. In addition to these valuable prehistoric contribution. The cyanobacteria have the potential to change the present agriculture world into bacterial world that naturally fix the nitrogen. Nitrogen is one of the essential entity for the growth of plants and their survival. Deficiency of this macronutrient in agriculture soils limits the growth and reproduction cycle of the plants in agriculture production. The nitrogen present in the atmosphere is in the molecular form and that cannot be directly utilized by most of the organism in nature. In order to utilize this molecular nitrogen, it must be fixed in the form of urea, ammonia, etc. To carry out this process a potential solution is cyanobacteria which are capable of fixing the atmospheric nitrogen. This present study focuses on one of the genes, called Nostoc. *Nostoc*, a genus of filamentous cyanobacteria. The filaments are typically arranged in beaded form to form a gelatinous colonies which is capable of fixing nitrogen and found globallyin both terrestrial and aquatic. From the previous studies, it is clear that some multicellular cyanobacteria such as Nostoc help in facilitating N2 fixation. Some filamentous cyanobacteria such as Nostoc (Fay, 1992) and Anabaena (Murry *et al*., 1984), develop specialized nonphotosynthetic cells, called heterocysts where nitrogen fixation occurs heterocyst is slightly larger than the vegetative cell. It is mainly concerned with the nitrogen fixation and sometimes reproduction too. The heterocyst has thick two layered envelope with inner walls forms knob-like projections into the cell cavity. Reproduction by heterocyst is rare but they germinate in certain cases. The heterocyst is liberated by breaking their walls. The nitrogen present in the heterocysts is transformed to the other vegetative cells along the filament, utilized by all population of vegetative cells (Flores and Herrero, 2005), in turn, it gets carbon resources a fully cooperative system (Wolk, 2000). An interaction mechanism between the vegetative and heterocysts is based on the current facts (Adams, 2000; Orozco *et al*., 2006; Zhang *et al*., 2006). Heterocysts developed from the vegetative cells of Nostoc, the visible

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changes during formation includes enlargement of a cell and accumulation of additional wall layers. A condition where the amount of N2 is limited, cyanobacteria produces heterocyst cells with nitrogenase which helps in N2 fixation (Berman-Frank *et al*., 2003; Bohme, 1998). The factors affecting N2 fixation in cyanobacteria are temperature and light (Gundale *et al*., 2012).

MATERIALS AND METHODS

The digital images that are required for this study are collected from Bharatidasan University, Tircinapalli, India. An experiment was carried out in by incubating the cultures in a growth room with a temperature of 27 degrees in BG-11 medium, 16 h light and 8 h dark over a period of 15 days. The images were captured with the help of microscope attached with a camera. Over 400 cells of this genera were collected and then the data is partitioned into training and testing data. Classification of algae images consists of different steps, namely, a preprocessing step, image segmentation, feature extraction and classification.

Preprocessing: Algal images that are used in many of the recognition systems are generated from microscopic images which contain different capturing information are used to create a database of the species (Fig. 1) (Mosleh *et al*., 2012; Persoon and Fu, 1977; Platt, 1999). This introduces a major problem in the processing of feature extraction from the captured images. In order to overcome we have preprocessing technique. Preprocessing is a process of making microscopic images of the species suitable for processing. First, the images are cropped by resizing them to 400 pixels and then the input color images into the gray-scale image. This is done because colors of different genera of algae may be identical as a result, in algae color feature is not suitable for detecting and identifying algae.

Image segmentation: Image segmentation is a method of extracting the object of interest from an image background. The main difficulty that we encounter is noise. Noise in an image is the unwanted objects which in turn disrupt segmentation process. One of the powerful tools for suppression of noise is the image smoothing. Segmentation is the fundamental need of any computer based vision application as it plays a vital role in processing and understanding of an image. The main motive of this technique is to simplify the representation of an image into something that is simpler to understand and represent. The main use of segmentation is to locate the objects, edges, lines in a digital image. Image segmentation is a method of labeling each of the pixels in an image in such a way that the pixels with the similar label share some visual characteristics. This methods output is a set of regions altogether serves as an entire image. There are the different technique to perform segmentation which is on the floor. The detailed analysis of image segmentation as per the literature works to identify a region, edges depends on a grouping of pixels which improves processing speed (Varshney *et al*., 2009; Tao *et al*., 2007). The various segmentation techniques help in improving the quality of recognition. Active contour models are used for the boundary refinement is an important process in segmentation. The segmentation technique which helps in a grouping of the pixels in an image led to some other techniques such as fuzzy based and K-means clustering process. The texture based encoding segmentation which emphasized in the reduction of coding and also maximum likelihood regions provides angular based patterns. The active contour

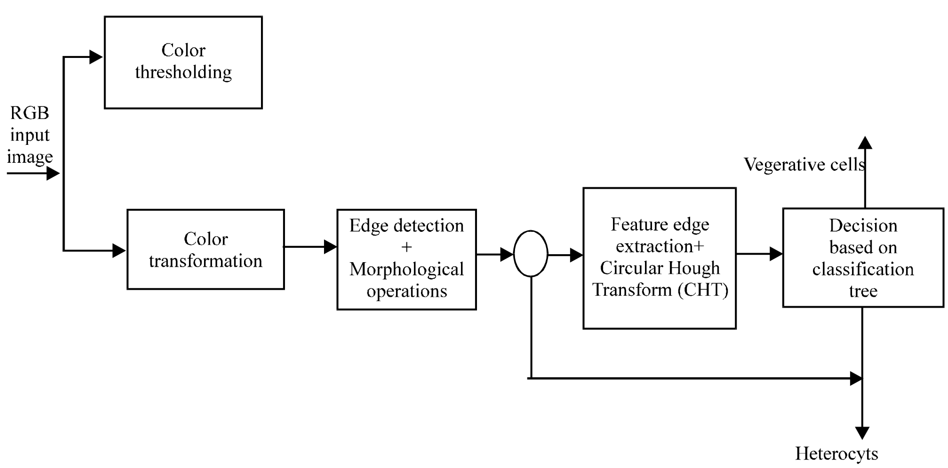

Fig 1: The proposed system

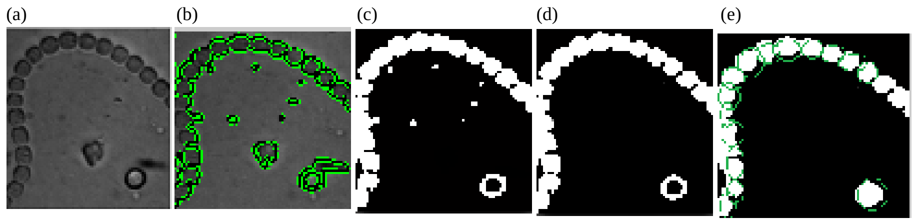

Fig. 2: An image segmentation method: a) Original image; b) Active contour image; c) Segmented image; d) Filling the boundary gaps and removing unwanted particles and e) Circular hough transform

segmentation which produces refinement of boundary and accuracy. In our study, we have made use of Chan-Vese Model (Sharifi *et al*., 2002) which is an unsupervised segmentation which analysis the local intensity and describes the region of an image.

The common problem faced by computer vision is to find the location, orientation of the object in an image. Hough Transform is used for finding any kind of shape. The equation of circular Hough transform represented using parameter space is given by the equation of a circle is Fig. 2. There are three different parameters in the given Eq. 1 where x_0 and y_0 are the centers of the forming circle along x and y-direction, respectively and 'r' is the radius. And x and y can take the values as:

$$X = a+r\cos(\theta) \quad (1)$$

$$Y = b+r\sin(\theta) \quad (2)$$

In this entire process of circular hough transform first to find all the edges in a given image. This first step is carried by making use of any of the edge detection technique of our choice like canny, sobel or any morphological operations (Rizon *et al*., 2005; Pedersen, 2007). After this step for each edge point we construct a circle with center and of the desired radius.

Feature extraction: Feature extraction is one of the important steps in the entire procedure in providing matching an image with different or unique information (Ruusuvuori *et al*., 2008). The common properties that can help in extraction the features are edges, points, intensity, texture and color are some of the properties of it (Rulaningtyas *et al*., 2011). These descriptors play an important role in both training and classification phase.

Pattern recognition: Pattern recognition is a machine learning which is an act deals with the raw data and the action performed based on few pattern categories (Richard *et al*., 2000; Yang *et al*., 2010). The different

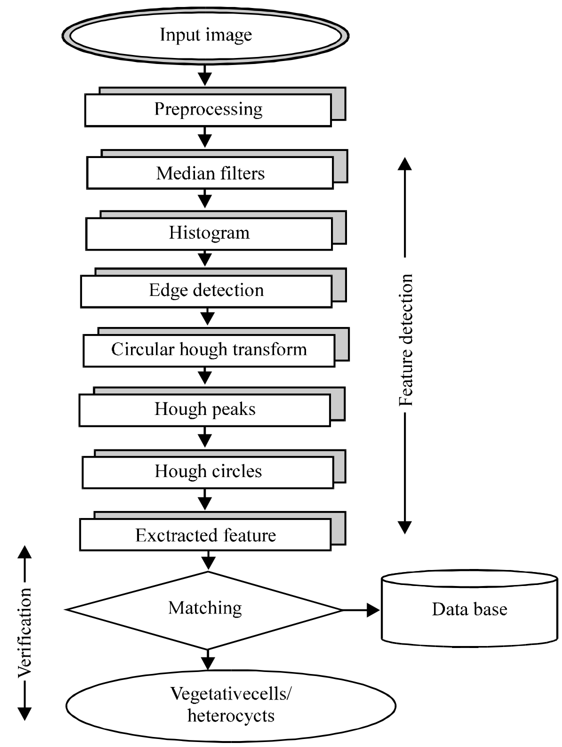

Fig. 3: Flow chart of proposed system

algorithms fall under pattern recognition composed of image segmentation techniques, feature extraction and classification (Yang *et al*., 2010). In this entire process first, the Nostoc image, segmented first and then the features are extracted from the Nostoc image. Then with the help of the training data set the classifier is trained by providing the extracted features are served as input the classifier.

Proposed system: The flowchart of the recognition process of the species is given in Fig. 3. Before the input image enters into the proposed system it has to undergo several steps. Image enhancement has to take place which is performed by using histogram equalization and other entities like removing unwanted objects from the image all these steps are carried in the preprocessing step. This histogram equalization helps us to increase the contrast of the image and helps in producing bitter results using region-based feature extraction. Among the various edge detection that is available, either Canny's or Sobel's edge detection techniques along with some mathematical morphological operations are applied to perform segmentation (Sharifi *et al*., 2002). Canny produces thin edges than compared to other edge detection techniques. Summarizing all the steps for CHT:

- Convert the colored image into grayscale
- Compute an accumulator a 3D hough array in which the first two dimensions represents the coordinates of the circle and the third dimension represents the radii
- Detect edges using Canny edge/Sobel edge detector
- For every edge, pixel Increment the corresponding elements in the Hough array
- Accumulate the circles generated using the hough array
- Construct the circles, around the Nostoc vegetative cells and heterocyst

Many morphological operations such as opening and closing are employed where the closing operation helps us to close the small gaps giving a clean and clear image. Whereas on the other hand, the opening operations help us to create small gaps and between the

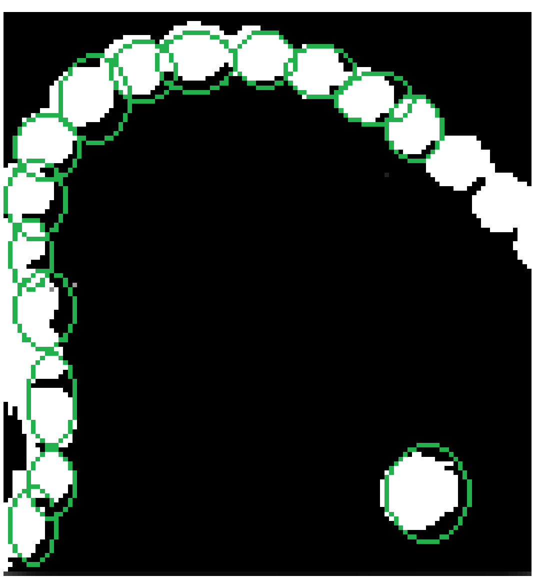

Fig. 4: Results of Hough transform

connected objects in an image. After all this now the segmented data is sent to CHT. When CHT is used, the radius 'r' is known in advance based on the object shape in the given image. So, radius 'r' can be used as a parameter. During the experiment, the object of interest is vegetative cells of Nostoc and the heterocyst (Fig. 4).

RESULTS AND DISCUSSION

In this automated system, the differentiating behavior of the vegetative cells and the heterocyst has been studied with the help of circular hough transform. An algorithm which is able to capture the complexity in finding the edges of each of vegetative cells present in Nostoc image and also the heterocyst in the proposed system. The specific characteristic behavior of Nostoc is observed by conducting experiments which can successfully differentiate the vegetative cells of the genera with heterocyst's present in the given image. The edge detection results used for segmenting the genera were not so promising as shown in Fig. 5. As per the experimental results the area enclosed within the vegetative cells is around 450 and heterocyst is around 573.

It is difficult to differentiate each vegetative cell present in an image as the separation of the edges is not proper. In order to overcome the difficulty in labeling the cells, CHT is applied on the segmented image by fixing the radius 'r' for each of the vegetative cell and heterocysts. By doing this the results obtained are accurate and most promising as shown in Fig. 2. Now, it is possible to label them. With the help of binary masking technique, the vegetative cells and the heterocysts are colored. For classification purpose, the geometrical feature such as area and number of pixels parameters are considered and based on these parameters, able to get the difference between the vegetative cells and heterocysts. To find the accuracy of the system a confusion matrix is created which is a table that describes the performance of a classification model or a classifier contains information about the predicted and actual on a set of data. The performance of the system is judged by using the dataset present in the matrix constructed by considering four values true positive, true negative, false positive and false negatives. The confusion matrix constructed for this study is shown in Table 1. The accuracy of the system is approximately calculated as 95%. By feeding the values of true positive, false positive, false negative and true negative and constructing the confusion matrix which calculates the accuracy of the system as 95% as shown in Table 2.

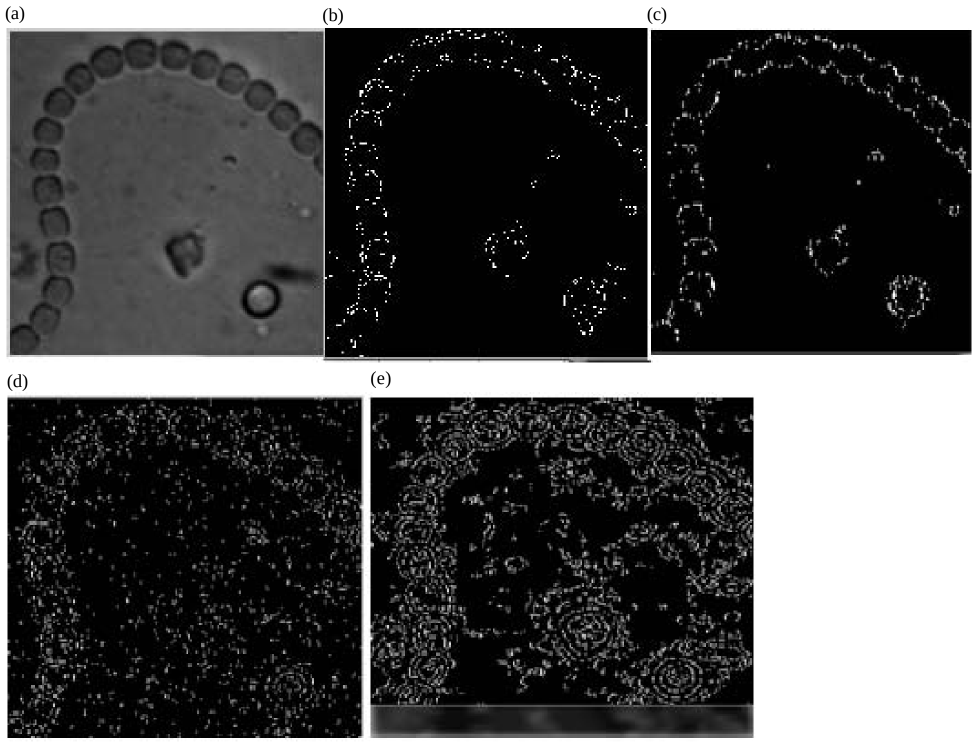

Fig 5: a) Original image; b) Prewitt; c) Roberts; d) Laplacian of a Guassian (LoG) and e) Canny

Table 1: Confusion matrix

N = 400	Predicted: No	Predicted: Yes	Values
Actual: No	TN = 70	FP = 14	84
Actual: Yes	FN = 7	TP = 309	316
	77	323	

Table 2: Different measures from confusion matix

Measures	Values	Derivations
Sensitivity	0.9778	TPR = TP/(TP+FN)
Specificity	0.8333	SPC = TN/(FP+NT)
Precision	0.9567	PPV = TP/(TP+FP)
Negative predictive value	0.9091	NPV = TN/(TN+FN)
Accuracy	0.9475	ACC=(TP+TN)/(P+N)

CONCLUSION

The confusion matrix helps to compute different measures such as sensitivity, specificity, precision, negative predictive value, false positive rate, false negative rate and accuracy.

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