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Digital Image Analysis of Algae Morphology During Lifecycle Using Active Contour

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Abstract: The main aim of the present study is to develop an automatic tool to identify and classify the growth stage of the microalgae cell based on morphological growth pattern and also the division time of the individual cell of microalgae population. The proposed strategy is to capture digital images of the microalgae cell growing on culture media and to examine the change in dimensions of each cell throughout life cycle. To identify geometrical features that are used in estimating the microalgae cell properties which are helpful for time determination during cell division. The segmentation method used, here is active contour and classification is done using fuzzy inference system and decision trees. The experimental results are compared with manual results obtained by phycologist and demonstrate the efficiency of the system.

INTRODUCTION

According to autecological studies, micro algal morphological growth in culture exhibits five different growth phases namely, lag phase, log phase, exponential phase, stationary phase and death phase. During the time interval of lag phase the algal cell will adapt themselves to growth conditions. This is the phase where individual algae cell is matured but not yet able to divide. During log phase and exponential phase this is a period which is characterized by cell doubling. The number of new algal cell appearing per unit of time is proportional to the present population. On account of rapidly deprecation of nutrients and accumulation of toxic, the exponential phase cannot continue indefinitely. During stationary phase, the growth rate slows as a result of nutrient depletion in the culture media. This phase exhibits constant value as the rate of algal growth is equal to the rate of algal cell death. The last phase is death phase, algae run out of nutrients and die[1]. The generation time is important in determining the amount of chlorophyll a, b, carotenoid, proteins, biomass during the different morphological growth phases. The algae cell growth cycle is shown in Fig. 1.

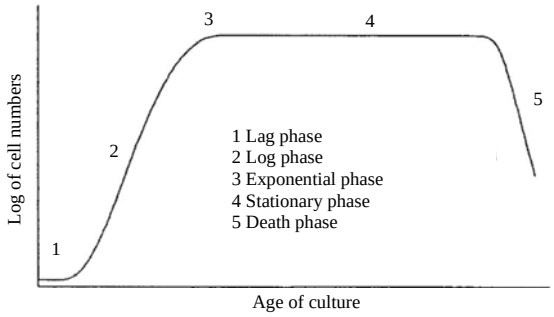

Fig. 1: Algal growth phases during the life cycle

For our experimentation, the micro algal cell images are captured at regular intervals in terms of days that are for cell division time determination. Form the literature survey it is evident the growth and the changes in

individual cell by image analysis using statistical analysis was investigated by Venkataraman[2]. Influence of environmental stress on distributions of times to first division in *Escherichia coli* populations is determined by using digital image analysis of individual cells. A analysis method for measuring lag times in division of individual bacterial cells using image analysis based on geometrical features has been done by Mata *et al*.[3], a novel method for measuring lag times in division of individual bacterial cells using image analysis. J. Microbiol. Methods 65, 311-317 (2005). Experimental study of cell shape and division in *Escherichia coli* has been carried out by Singh *et al*.[4]. Automated identification and classification of bacilli bacterial cell growth phases based on geometric features by Spolaore *et al*.[5].

The characteristics that need to be considered and studied in the present work are the size of the cells before and after division, the variation in the generation time of algal cells, spatial and successive change in morphology of the individual cell size. The factors that plays a vital role in changes in morphological growth phases during the life cycle of micro algae are temperature, light, pH and medium components. By change in any of the above factor shows the changes on the morphological patterns of the cell in each of the growth phases[6].

MATERIALS AND METHODS

This present study was performed on *Pediastrum* sp., a green alga predominant in freshwater. These *Pediastrum* sp. colonies are commonly found in the form of a colony, disk-shaped and are characterized by peripheral hornlike projections. Depending on the species the cells present per colony varies in between (2-7). These samples are collected from Bharathidasan algal depository for our study. These species are studied under a confocal microscope attached to a camera, at an optimal temperature of 25°C and pH of 7.5 with dark and light cycles.

Proposed system: An active contour is essentially a curve made up of various energies. The curve deforms dynamically to the shape of a targeted object. There are various methods for implementing an algorithm to achieve object outlining. The energies in the active contour can be divided into two categories. Internal and external energy functions which have the relationship are discussed. The Internal energy functions focus on the intrinsic properties of the contour such as elasticity and curvature while the external energy functions are related to the image properties like contrast and brightness. Esnake = Einternal+Eexternal.

Active contours or snakes are computer-generated curves that move within images to find object boundaries. They are often used in computer vision and image analysis to detect and locate objects and to describe their shape. For example, a snake might be used edge detection, corner detection, motion tracking and stereo matching; one might be used to find the outline of an organ in a medical image; or one might be used to automatically identify characters on a postal letter. A snake is an energy-minimizing spline guided by external constraint forces and influenced by image forces that pull it toward feature such as lines and edges. Snake are active contour models: they lock onto nearby edges, localizing them accurately. Scale-space continuation can be used to enlarge the capture region surrounding a feature. Snakes provide a unified account of a number of visual problems, including detection of edges, lines and subjective contours; motion tracking and stereo matching. The basic snake model is a controlled continuity spline under the influence of image forces and external constraint forces. The internal spline forces serve to impose a piecewise smoothness constraint. The image forces push the snake toward salient image feature like lines, edges and subjective contours. The external constraint forces are responsible for putting the snake near the desired local minimum. These forces can, for example, come from a user interface, automatic attentional mechanisms or high-level interpretations[7, 8].

Representing the position of a snake parametrically by v(s) = (x(s), y(s)), to obtain the best fit between the snake and the object, we minimize the energy. Specifically, a snake is defined as:

$$E^*_{snake} = \int_0^1 E_{snake}(v(s))ds$$
$$= \int_0^1 E_{int}(v(s))ds + \int_0^1 E_{image}(v(s))ds + \int_0^1 E_{forces}(v(s))ds$$

Where:

E_{int} = The internal energy of the spline due to bending
E_{image} = Gives rise to the image forces and gives rise to the external constraint forces

The internal energy provides a smoothness constraint. This can be further defined as:

$$E_{int} = \alpha(s)\left|\frac{dv}{ds}\right|^2 + \beta(s)\left|\frac{d^2v}{d^2s}\right|^2$$

The spline energy is composed of a first-order term controlled by α(s) and a second-order term controlled by β(s). The first-order term makes the snake act like a membrane and the second-order term makes it act like a thin plate. Adjusting the weights α(s) and β(s) controls the relative importance of the membrane and thin-plate terms. Image gradient and intensity are obvious (and easy) characteristics to look at (another could be object size or shape). Therefore, the following external energy function is investigated:

Table 1: Decision rules to determine morphological growth phases during the life cycle of *Pediastrum* sp.

Inputs		Decision	
Range of area values	Time of division in terms of days (approx.)	Cell growth phases	Cell division determination
4000-4700	1-3	Lag phase	Normal
4800-5800	4-7	Lag phase	Grown up
6000-37500	8-22	Log phase/Exponential phase	First division
40000-57000	22-29	Stationary phase	Second division
57000-58000	29-32	Stationary phase/Death phase	Final division

$$\beta E_{ext}(v_i) = mE_{mag}(v_i)+gE_{grad}(v_i)$$

Where:

$E_{may}(v_i)$ = An expression that attracts the contour to high or low intensity regions

$E_{grad}(v_i)$ = An energy term that moves the contour towards edges

Again, the constants m and g are provided to adjust the relative weights of the terms. The total image energy can be expressed as a weighted combination of the three energy functionals:

$$E_{image} = \omega_{line}E_{line} + \omega_{edge}E_{edge} + \omega_{term}E_{term}$$

The present study is developed to automate a method to determine the different cell growth morphology during different phases in the life cycle of cyanobacteria and also to know time of individual cell of an algal population. Based on determining geometrical feature and statistical features. The geometrical feature considered for this experimental study are length, breadth, area, perimeter, circularity, entropy and Box Area Ratio (BAR)[9, 10].

Training and classification: The proposed study to detect and identify different morphological stages of an algal image and also time determination of algal cells captured at regular intervals (in terms of number of days) based on their geometrical and statistical features values.

Algorithm 1: During training phase (Segmentation and feature extraction of cell regions).

1. Input an algal digital cell image
2. Pre-processes the image by using morphological operations like, filling, erosion, dilution, area open
3. Segment the input digital image using active contour or snake
4. Label the segmented image, where individual segment is known as cell region
5. For each of segmented cell in an image compute the BAR value and area, perimeter, circularity, eccentricity, centroid
6. Repeat the above procedure from 1-5 for all cell images taken on regular interval in terms of number of days namely 1,3,5,7, so on 30 th day and store them as knowledge base

Algorithm 2: Classification phase.

1. Input an algal digital cell image
2. Pre-processes the image by using morphological operations like, filling, erosion, dilution, area open
3. Segment the input digital image using active contour or snake
4. Label the segmented image, where individual segment is known as cell region
5. For each of segmented cell in an image compute the BAR value and area, perimeter, circularity, entropy
6. Repeat the above procedure from 1-5 for all cell images taken on regular interval in terms of number of days and store them as knowledge base
7. Apply the decision rules, for all test cell images captured at regular number of days for determination of the cell growth phases, namely, lag, log, exponential and stationary and death phases cell division times corresponding to initial division, second division, etc., based on the values obtained from Area computed with respect to each segmented cell in an image for different phases. Decision rules

Decision rules: The decision rules for the classification algorithm is obtained from section 3.1 by taking the training data set:

Based on the cell division time in the lag phase, say if the value of the Area of each cell is in between 4000 and 4700 then that cell belongs to lag growth phase it is normal cell[11].

If the Area value of the cells is 4800 and 5800 then this particular cell is in the lag phase but it is in about-to-divide (grown up) stage.

If the area value of the individual cell lies in between 6000-37500 then the cell is in log or in exponential stage. Which indicate that the single cell has undergone division. If the Area value of the individual cell lies in between 40000-57000 then the cell is stationary phase and the cell division time is (25-30th day)[12].

The above decision values are summarized in Table 1. Table 1 contains the information about different range of area values at a given time interval in term of number of days which corresponds to different morphological growth phases obtained from training images and stored in the knowledge base. These above rules that are present are helpful in the fuzzy inference rules for classification system.

RESULTS AND DISCUSSION

In the present study, we have considered *Pediastrum* sp. cyanobacteria cell images which exhibits different growth phases based on morphology chaptered. The 300 sample are considered to carry out the experiment. The image is size is of 350×350. The data set contains 90, 80 and 70 images corresponding to lag, log/exponential and stationary phases. Among the entire data set 50% is

Table 2: Classification accuracy

Confusion matrix	Initial stage	Second stage	Third stage	Final growth phase
Initial stage	296	4	0	0
Second stage	1	298	1	0
Third stage	0	0	294	6
Final growth phase	0	0	1	299

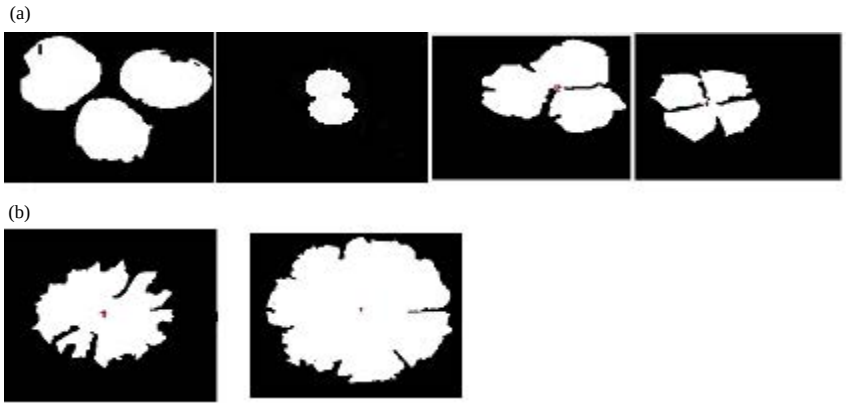

Fig. 2(a, b): Segmented images of *Pediastrum* sp.

chosen as training set and remaining 50% as testing dataset from Fig. 2a. It is observed that the sample of *Pediastrum* sp. cell images captured and shows different morphology in growth patterns and subsequently the corresponding segmented images is shown in Fig. 2b. It is observed that, the first division of *Pediastrum* sp. on BG-11 at 25±2°C, after a duration of 3 days the cells of this species in normal stage. This is lag phase as shown in Table 1. After this the normal cell began to grow and get divided, this indicates cell is moving from lag phase to log/exponential phase the time taken to attended this phase is approx. from fifth day to 21 st day depending on species under consideration *Pediastrum* sp. these cells again increase in their dimension multiples at a faster rate. When the cell entire in third phase, i.e., stationary phase it stops dividing and remain in the same state and then later as the nutrients in the medium degraded the cell slowly moves towards the death stage[13-15].

Fuzzy inference classification system: Computing the mean and standard deviation of the training data set. The Sugeno model is used to model any inference system in which membership function is either linear or constant[16]. The fuzzy membership functions for area corresponding to lab, log/exponential and stationary phases are shown in Fig. 2 where the lag phase is divided as early and late lag phase, then followed by log/exponential phase and stationary phase. Mean and Standard deviation of BAR values computed for an individual cell captured at regular intervals w.r.t days is shown in Table 2. Classification accuracy of proposed method for *Pediastrum* sp. cyanobacterial morphological cell growth phases is as shown in Table 2[17].

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CONCLUSION

We introduced an algorithm for *Pediastrum* sp. microalgae classification. The segmentation algorithm is composed of a histogram equalization, color quantization and active contours iterations. The active contours algorithm shows results close enough to the manual segmentation procedure. The feature extraction to the segmented and original images. The classification is realized with rule based and fuzzy machines classifiers. The rule based classifier shows better results with respect to the fuzzy classifier. We reach 98.63% of performance with rule based, ground truth images and a mixture of features with SFS. The database derived from this work is publicly available for download. A total of 1200 images containing examples of 24 microalgae representative of the major algal phyla was processed and the correct number of database classes and the correct assignment.

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