STUDY OF SCENEDESMUS ALGA GROWTH VIA IMAGE ANALYSIS *

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Abstract

This paper is focused on applying image analysis algorithms in biological field. Nowadays, all the modern measurement equipments produce a large amount data in digitally form including microscopes. Developing of automatic or semi-automatic tools e.g. in Matlab for various tasks could be helpful. The algorithm set described in this paper analyze a large sequence of microscope images of *scenedesmus* growth and evaluate quantitative features. Within developing this set, a novel segmentation algorithm which is based on artificial neural networks was proposed. This algorithm highly outperform the classical image processing approaches.

1. Introduction

Scenedesmus is genus of colonial green algae mainly with 4 (rarely 2, 8, or 16) cells arranged in a row [1]. A common component of freshwater plankton, it is used in experimental work on photosynthesis and for artificial cultivating. It has been added to feed (fishes, stock as well as humans) because it contains valuable substances (e.g. antioxidants and unsaturated fatty acids). Algae scientist are mainly interested in a colony behavior and in a matter how the cells influence each other. The visual analysis of one colony growth under a microscope could be helpful. Doing this analysis by hand is a painful work because it is necessary to observe many colonies for a long time. Nowadays, the microscopes are equipped by digital cameras which are programmable and it is not a problem to capture the whole colony growth into a computer fully automatically. Computers can analyze the pictures sequences also. A set of algorithms described in this paper is one eventuality how help to study the *scenedesmus* growth via automatics image analysis.

2. Algorithms description

The goal of the set of algorithms was find the colony of cells in the pictures, track the cells and quantitatively evaluate the size of cells and its inner organelles during whole growth until the cells are spitting into two new colonies. The images from sequence is processed independently and from the algorithmic point of view, the process could be divided into following steps:

- distinguish objects and background in picture
- find the colony between detected objects
- classify inner cells structures
- evaluate quantitative features

2.1 Source data description

The cells were observed by inverted microscope OLYMPUS IX51. Every 2 minutes, one image was taken by camera OLYMPUS C-7070. Resolution of the images was RGB 7Mpx (3072*2304 pixels), images were stored in JPG format. The image sequence length depends on moment, where the cells split. It takes approximately 4 hours. It means that the sequence has about 120 images/frames. The input images was resized by factor 0.2 before processing for faster computing.

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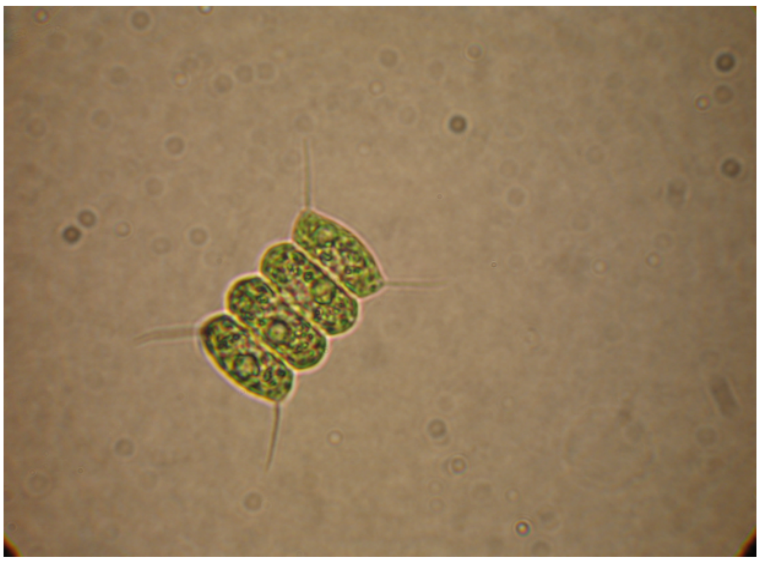

Figure 1: An example of input image (cut-off)

2.2 Step 1 – distinguish objects and background

At first, an appropriate thresholding method has to be applied. In initial experiments, commonly used methods were tested. The RGB domain for thresholding is not advisable, because objects and background are inseparable in this domain. HSV [2] and chromatic colors [3] domains were tested instead. The image in HSV domain was obtained by function *rgb2hsv* (from Matlab Image Processing Toolbox). The image in chromatic colors was obtained by using equations (1).

$$C_r = \frac{B}{R+G+B}$$
$$C_g = \frac{G}{R+G+B} \qquad (1)$$
$$C_b = 1-(C_r+C_g)$$

The C_b channel in chromatic color domain is redundant because it is linear combination of C_r and C_g. The thresholding itself was done by *Otsu* segmentation method [4], which is a gray level thresholding nonparametric method of automatic threshold selection for picture segmentation from intensity histogram. The *Otsu* segmentation was done in all channels HSV and C_r,C_g, respectively. The final binary result was obtained by logical AND operation of individual channels. Both domains get very simmilar results but quality of these results was practicaly on the threshold of usability for next processing because a quality of input images are far from ideal state. The morphologic operation can help a lot but are not able to correct all errors. After the initial tests with above described method, it was clear that a new more powerful thresholding method have to be developed.

The new developed method is based on artificial neural networks (ANN) with using Matlab Neural Networks Toolbox. In training stage, one ideally segmentated binary image was prepared together with an input image (in some domain RGB, HSV or chromatic colors). The ideally segmentated image could be prepared by hand or semiautomatic (via old version of segmentation method and via morphological operations with well tuned parameters). ANN could be trained by this data. The appropriate network topology is a two layers non-linear network with *tansig* transfer functions. Number of neurons in hidden layer was set to the input dimension. More neurons could be used but this number was sufficient and no significant performance gain was obtained by higher numbers of neurons. Output of ANN is one

dimensional and its *tansig* function produces values in range <*-1,1*>. Pixels where the output value is greather than zero is marked as object, less than zero marked as background. Performance of ANNs was evaluated via dichotomic classification task on a few others ideally segmentated images. The performance of ANNs varied on used input domain. RGB domain gave the worst result: 92% correctly classified pixels. Chromatic colors domain occupied the second place with 94.5%. The best of was HSV domain with 95%. For comparision, the old algorithm was evaluated as well. The result was 91%.

The produced errors were analyzed. The most errors were done by the missclassification of others blured small objects in captured sample. From this reason, the strategy of ANN using was changed. The segmentation proces was splited into two tasks. The first task is distiquish the all object from the background. The second task is distinquish the *scenedesmus* from all other extraneus object in the image. The two-stage process is shown in Figure 2. This two-stage method enhances performance to 98% in HSV domain. This much better segmentation method was applied as a preprocessing step in all alghorithms described bellow.

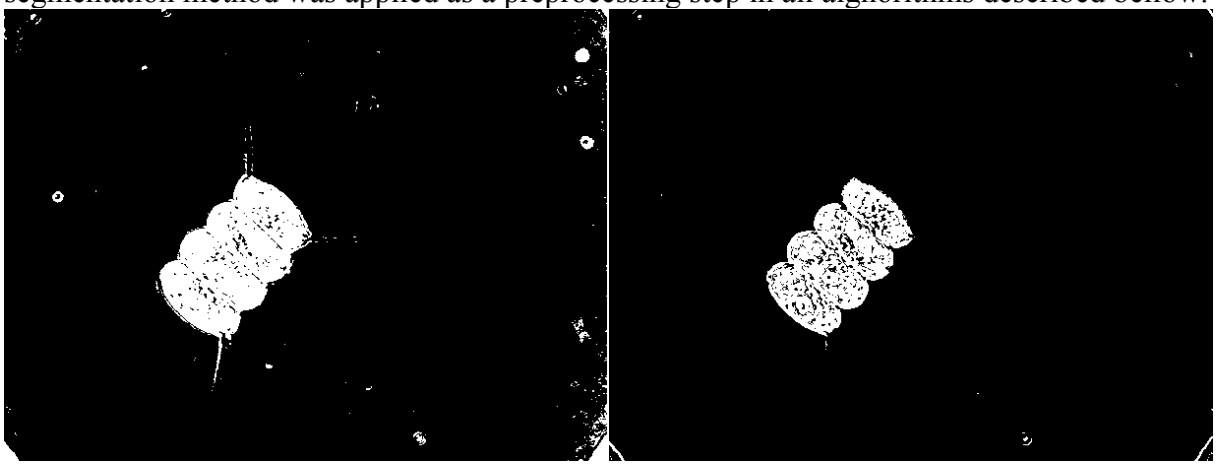

Figure 2: Illustration of two-stage ANN segmentation process.

Next, the initial segmentation generated by two-stage ANN process was enhanced via morphologicals operations. The following steps were applied:

- fill holes in objects - `imfill(bw, 'Holes')`
- clear borders - `imclearborder(bw)`
- close - `imclose(bw, selement(5))`
- open - `imopen(bw3, selement(7))`

The our-own `selement` function gives a circular structural element matrix with diameter 5 or 7 pixels. After all this steps, the clear image where ussualy only the *scenedesmus* objects left is obtained (Figure 3).

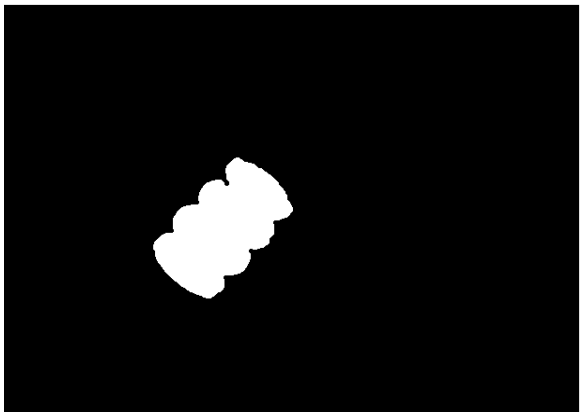

Figure 3: Final binary image after segmentation.

2.3 Step 2 – find colony objects

This step are relatively easy to do because the segmentation produces very good results and in the image are only a few extraneous object or no one. Classification of the objects are based on its size/area. Two approaches could be used: The first one keep only these object which have a size in a priory defined range typical for *scenedesmus*. The second one need information about expected number of *scenedesmus* colonies in image. Then select the same number of object which have the size closest to the typical *scenedesmus* value. If the information about number of colonies is not available than the first one approach is used. In all our tests, the first one approach was sufficient.

2.4 Step 3 - classify inner cells structures

The *scenedesmus* cells contain a large number of different inner structures but it is impossible to distinguish all from each other only via processing of these low resolution images. From this reason, the inner objects were divided into three classes which are visually separable: Transparent border membranes (***C1***), inner cytoplasm (***C2***) and inner organelles like e.g. chloroplasts, mitochondria, endoplasmic reticulum and nucleus (***C3***). The classification algorithm is based on minimum euclidean distance in RGB space. Centroids for the classificator were trained by unsupervised k-means method. An illustration of the results is depicted in Figure 4.

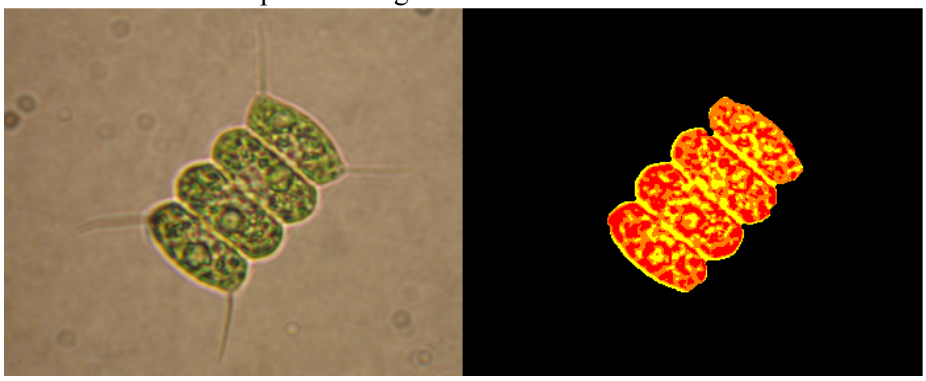

Figure 1: Illustration of image processing, inner structures of cells on input image (left) are classified into three classes (right).

2.5 Step 4 - evaluation of quantitative features

The *scenedesmus* growth were analyzed via evaluation areas of individual classes. Together with the areas trends, the areas ratios between individual classes are interesting also. For future analysis, the summed areas were evaluated as well as areas of individual cells were evaluated. Example of summed areas trends are depicted in Figure 5.

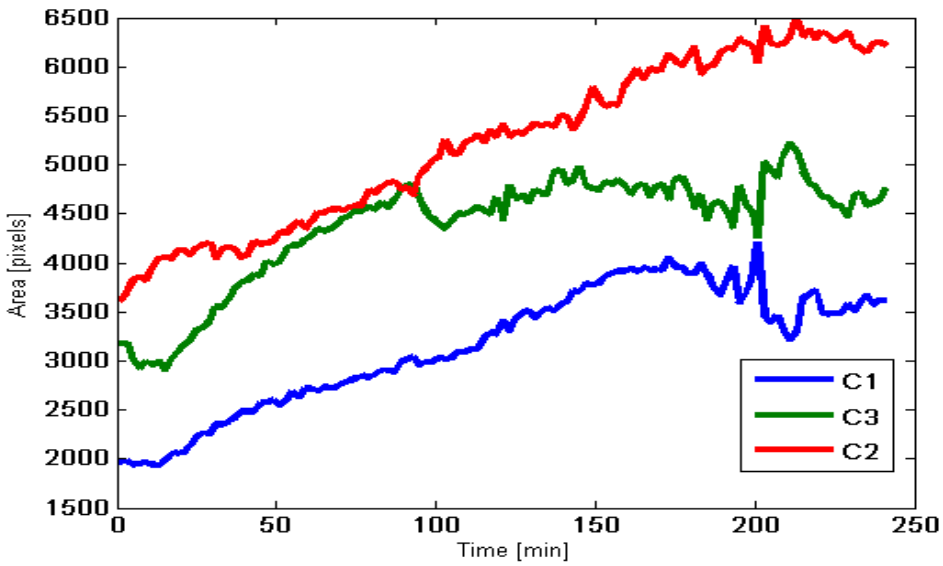

Figure 5: Trends of summed areas of individual classes.

Conclusion

In this paper, an algorithm set which is able to help with study the *Scenedesmus* growth was described. The novel method of very powerful segmentation was described in detail in section 2.2. Source codes for using ANN for segmentation is included in electronic form. The quantitative evaluated features are an example how kind of information could be extracted. More useful information can be evaluated but a decision which features could be interesting depend on the focus of individual biological tasks.

References

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