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A Study on the Automatic Counting Method of Number of Chlorella

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Abstract: Marine chlorella (*Nannochloropsis oculata*) is cultivated at the fish farming association of experimental fishery laboratory. In the culture of the chlorella, it is necessary to count the number of chlorella in order to understand the condition of increase. For that purpose, we develop automatic chlorella counter using the image processing and the neural network. Its effectiveness is confirmed through the experiment.

1. Introduction

Marine chlorella (*Nannochloropsis oculata*), a kind of phytoplankton, is cultivated in open-air condition and is fed to zooplankton, and zooplankton is fed to fluke at the fish farming association of experimental fishery laboratory in Tomari Village, Tottori, Japan. For nursery cultivation of a fluke, chlorella is cultivated.

In the culture of chlorella, it is necessary to count the number of chlorella in order to understand the condition of increase. For that purpose, under the present state, there are three methods such as, by the naked eye using the microscope, by using colony counter and by using Coulter counter. Colony counter is the equipment using the image processing for automatic counting. But it is required that setting the condition by human, and the result fluctuates according to the setting of the microscope. Coulter counter is a high-performance equipment and uses change of electric resistance between the electric poles. The result is more accurate than that of colony counter, but the equipment is expensive. The fundamental method using the naked eye requires a lot of time and work.

In this paper a new technique of the image processing using neural network for counting the number of chlorella efficiently is proposed as solution of the problem as mentioned above. The algorithm for counting the chlorella is developed and its effectiveness is confirmed through the experiment.

The treated images in this paper are taken in slightly different condition such as different brightness and blurring by out of focus.

In reference [1], abnormal red and white corpuscle etc. into urinary ingredient are classed as an example that neural network is utilized for classification of microscopic object like chlorella.

The purpose of using neural network is imitating human ability by flexibility of neural network and setting no threshold of separating patterns. Then, proposed method can deal with the treated images in this paper. Patterns are classified into "chlorella" and "non-chlorella". For instance, microorganisms, bubble, dead chlorella, impurities into seawater and so on are not chlorella.

Additionally, revising the level and normalizing the level and the size is characteristic of proposed method by paying attention to that the color of chlorella is green and chlorella that is nearly a circle in two dimensions.

2. Proposed Method

Automatic counting method of number of chlorella is proposed as shown in Fig. 1. The automatic counting result of number of chlorella is obtained by proposed method. The procedure is as follows.

(I) Image of the chlorella is taken by the digital video camera from the eyepiece of the microscope, and stored in the personal computer. The stored color image is the input.
(II) In order to decrease the data amount, input color image is converted to monochrome image which has only element of green, represented as G monochrome hereafter. Here, only the element of green is used by paying attention to that the color of chlorella is green.
(III) Averaging filter is applied to G monochrome. Then, the image of an object is prevented from separation by binarization. Additionally, edge of an object as connected component becomes smooth, and background noise is also removed.
(IV) Binarization using Otsu's automatic threshold selection method [2] is applied to the blurred G monochrome.
(V) Labeling is applied to the binary image for classification of each connected component. The number of connected component is obtained by labeling. In the labeling, the number is orderly allotted to each of the connected component as 1,2,3,··· [3].

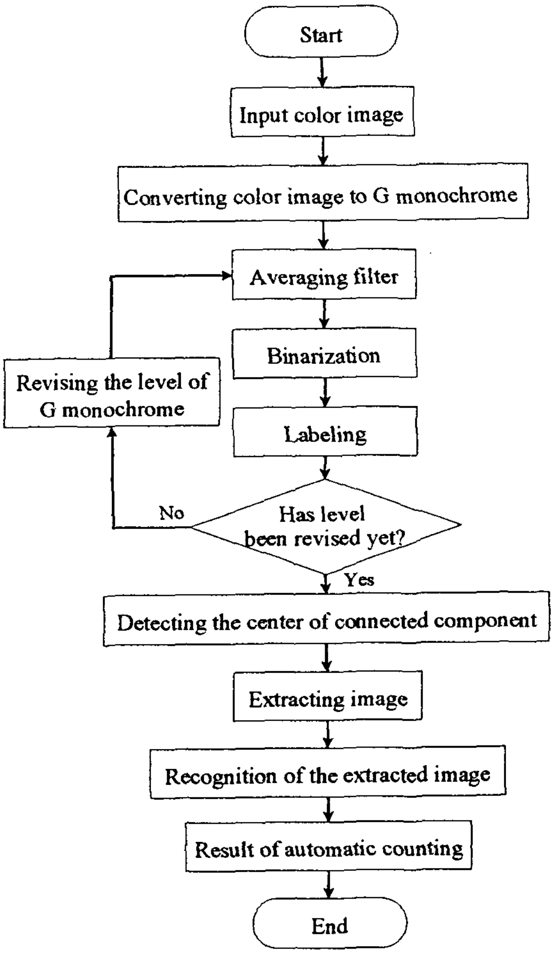


Fig. 1 Algorithm of proposed method

(VI) Compactness, which is circularity in the field of image processing, of each connected component is calculated, and the level of G monochrome is revised by the rate of number of inferior compactness. After calculating perimeter and area of each connected component, each compactness will be given by

$$Compactness = 4\pi \times (Area) / (Perimeter)^2 \tag{1}$$

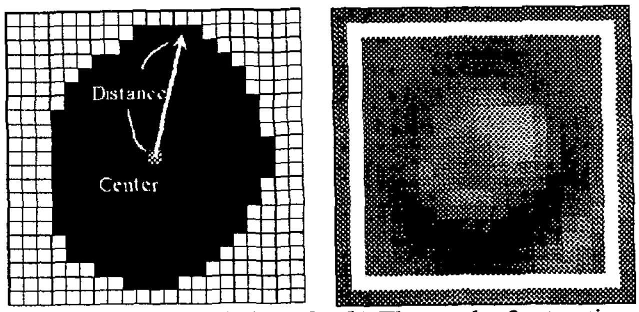
where perimeter is represented by Euclid distance.

Chlorella is more easily detected by being darkened level of G monochrome, because the color of chlorella is green. Chlorella is nearly a circle in two dimensions since chlorella is nearly a sphere in three dimensions. After revising the level of G monochrome, averaging filter, binarization and labeling are applied to revised G monochrome in due order.

(VII) The center of the labeled connected component is calculated utilizing the characteristic of the moment.

The moment is one of the characteristics of the shape of figure. It is determined by $M(p,q)$ is defined by

$$M(p,q) = \sum_{(i\,j)} i^p j^q f_{ij} \tag{2}$$



(a) Concept of calculation of extracting range
(b) The result of extracting (Extracted image is 17 × 17 pixels)

Fig. 2 Example of extracting image

where the image of $i \times j$ pixels is considered, $\{f_{ij}\}$ is a binary image of the labeled connected component. The center of gravity (I_c, J_c) is given by

$$I_c = M(1,0)/M(0,0) = \sum_{(i\,j)} i f_{ij} \Big/ \sum_{(i\,j)} f_{ij} \tag{3}$$

$$J_c = M(0,1)/M(0,0) = \sum_{(i\,j)} j f_{ij} \Big/ \sum_{(i\,j)} f_{ij} \tag{4}$$

(VIII) From the whole G monochrome image, candidates of chlorella are extracted as a square whose center is the center of gravity of the labeled connected component.

After calculating average of Euclid distances from the center to outermost outline of connected component, the extracted range is determined by being based on the average. For that reason, chlorella is nearly a circle in two dimensions. Figure 2 (b) shows an example of extracting image. The range of extracting is 17 × 17 pixels enclosed by the white line.

(IX) The extracted G monochrome images are the candidates of chlorella. Each of them is judged whether it is a chlorella or non-chlorella by the previously learned neural network. The neural network is explained in the following chapter.

Size of the extracted image is normalized after normalizing level of the extracted image.

Normalization of level is applied for canceling influence of brightness [4]. Extracted image whose size is e^2 pixels is converted to one-dimensional data by horizontal scanning, and is shown by vector x, as follows.

$$\vec{x} = \left[x_1, x_2, \cdots, x_{e^2}\right]^T \tag{5}$$

Then, the image after normalizing level is expressed by

$$\vec{x}_d = \vec{x} \Big/ \sqrt{\sum_{i=1}^{e^2} x_i^2} \tag{6}$$

Normalization of size is applied for revising irregular size of chlorella and adjusting a little difference of magnification in filming original image. Additionally, pattern recognition using neural network is not suited for such irregular size. Size of the extracted image is normalized regular size by using affine transform as liner transform.

3. Neural Network

A neural network is employed for judging whether the extracted G monochrome image is chlorella or non-chlorella. The neural network in this paper has 3-layer including one hidden layer as shown in Fig. 3. BP (Back Propagation) learning algorithm [5], [6] is employed. In a 3-layer neural network, unit of neighboring layer is combined each other, but each unit in the same layer is not combined.

If the pattern P is given to the input of the neural network, output of each unit of the k th layer is given by

$$o_{Pj}^{k} = f\left(\sum_{i=1}^{N_{k-1}} w_{i,j}^{k-1,k} o_{Pi}^{k-1} + \theta_j^k\right) \quad (7)$$

$$f(x) = \frac{1}{1+\exp(-\varepsilon x)} \quad (8)$$

where $o_{Pj}{}^{k}$ is an output value of unit j of the k th layer when pattern P is applied to the input. $w_{i,j}{}^{k\text{-}1,k}$ is the weight from the i th unit of the k-1 th layer $u_i^{k\text{-}1}$ to the j th unit of the k th layer u_j^k. $\theta_j{}^k$ is the offset of the j th unit of the k th layer. N_k is number of the k th layer. ε is the slant of sigmoid function.

In BP learning algorithm, as the cost function of learning of the neural network, target output and the sum of least mean square E_P is given by the following equation.

$$E_P = \frac{1}{2}\cdot\sum_{i=1}^{N_n}\left(t_{Pi} - o_{Pi}^{n}\right)^2 \quad (9)$$

where t_{Pi} is a target output to the i th unit of the last layer when the pattern P is applied to the input.

The value of the weight for update and the value of the offset for update are given by

$$\begin{cases} \Delta_P w_{i,j}^{k-1,k}(m) = \eta\delta_{Pj}^{k} o_{Pi}^{k-1} + \alpha\Delta_P w_{i,j}^{k-1,k}(m-1) \\ \Delta_P\theta_j^k(m) = \eta\delta_{Pj}^{k} + \alpha\Delta_P\theta_j^k(m-1) \end{cases} \quad (10)$$

$$(k = 1, 2, \cdots, n)$$

where

$$\delta_{Pj}^{n} = \varepsilon\left(t_{Pj} - o_{Pj}^{n}\right)o_{Pj}^{n}\left(1 - o_{Pj}^{n}\right) \quad (11)$$

and

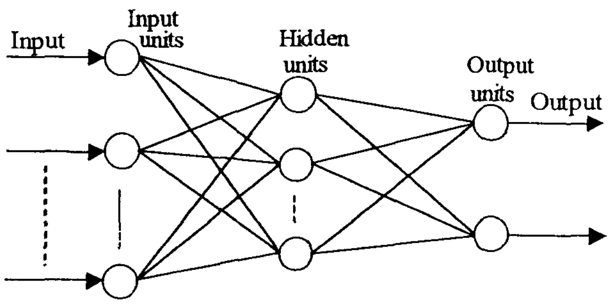


Fig. 3 3-layer neural network

$$\delta_{Pj}^{k} = \varepsilon o_{Pj}^{k}\left(1 - o_{Pj}^{k}\right)\sum_{s=1}^{N_{k+1}}\left(\delta_{Ps}^{k+1} w_{j,s}^{k,k+1}(m-1)\right) \quad (12)$$

$$(k = 1, 2, \cdots, n-1)$$

$\Delta_P w_{i,j}{}^{k\text{-}1,k}$ is value of the weight $w_{i,j}{}^{k\text{-}1,k}$ for update when pattern P input. $\Delta_P\,\theta_j{}^k$ is value of the offset $\theta_j{}^k$ for update when pattern P input. η is parameter of learning. α is parameter of momentum. m is iteration.

The neural network is trained until the following value E becomes smaller than the regulation value.

$$E = \frac{1}{C}\sum_P E_P = \frac{1}{2C}\sum_P\sum_{i=1}^{N_n}\left(t_{Pi} - o_{Pi}^{n}\right)^2 \quad (13)$$

where C is a number of patterns.

4. Experimental Results

The software of counting number of the chlorella is developed by C that is one of the programming languages.

The condition of experiment is as follows. The size of input color image is 630 × 480 pixels, and elements of RGB (Red, Green, Blue) are 8bit, respectively. The size of window of averaging filter is 3 × 3 pixels and the filter is applied twice. The size of the normalized extracted image is set to 11 × 11 pixels.

BP learning algorithm is employed for a 3-layer neural network, which has 121 units at the input layer, 80 units at the hidden layer and 2 unit at the output layer. Number of unit in the input layer is set to 121 by considering the number of input one-dimensional data of the normalized extracted image. The one unit in the output layer is used for "chlorella", and the other for "non-chlorella". Then, larger output value is the result of judgement whether it is chlorella or non-chlorella. Hence, the threshold of separating patterns is unnecessary.

In BP learning, if the value E in Eq. (13) becomes under 0.005, training will be finished. 25 images of the chlorella and 15 of the non-chlorella are used as the training data. The level and the size of these training data are normalized.

When we apply the color image, Fig. 4 and Fig. 5 are obtained. Figure 4 shows the candidate of chlorella. The inside of the white square is extracted. The size of extracting is different for each object. The extracted image is judged whether it is the chlorella or non-chlorella by the neural network. Figure 5 is the result of judgement, where the white point indicates that the candidate is chlorella.

Experimental results are shown in Table 1. The result of counting chlorella in monochrome image by author is also presented. The number of error is the number of non-chlorella in the output of our proposed method.

Final result of number of chlorella is the difference between the number of counting by proposed method and the number of error. The recognition rate is given by the number of chlorella divided by the number of counting by human.

In input image A, the color of chlorella is pale, in input image B, it is a little pale and in input image C, it is a little dark. Here, standard of the level is detected by author. Shading is caused by the light of microscope in filming original image. Input image D is nearly equal to input image E, but there is the difference of focus. Input image E is whose color is pale and chlorella is blurred by a little out of focus. In filming original image, if the focus is changed, brightness is also changed. The case of input image F, recognition rate is poor, because this image is rather vague to judge candidates of chlorella even by author correctly.

The experimental results show that the number of chlorella can be counted automatically even if the images are taken in different conditions.

5. Conclusion

In this paper, the chlorella counter using the image processing and the neural network has been examined. It has been confirmed by experimental results that the high recognition rate could be obtained. Proposed method could deal with uneven size and level of chlorella and the images that are taken in a little different condition. Then, proposed method is effective for the realization of automatic counting system of chlorella.

The improvement of revising of level and solution to the contact of chlorella are left for further works.

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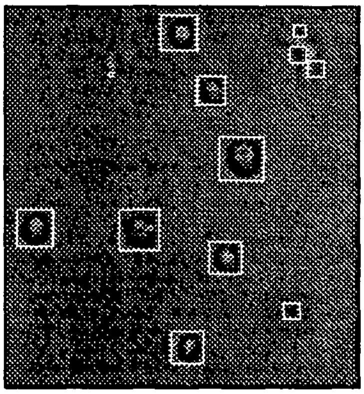

Fig. 4 Candidate of chlorella
(175×190 pixels)

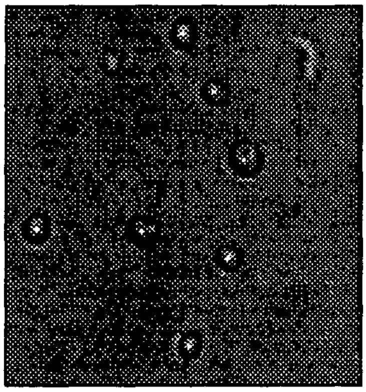

Fig. 5 Output of proposed method
(175×190 pixels)

Table 1 Experimental results

Input image	The number of chlorella by human	The number of chlorella by proposed method	The number of error	Recognition rate (%)
A	42	42	1	97.6
B	38	38	1	97.4
C	68	64	0	94.1
D	39	39	0	100.0
E	40	40	0	100.0
F	88	78	0	88.6