Automatic Identification of Diatoms with Circular Shape using Texture Analysis

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Abstract—**Diatoms are unicellular microscopic algae found in practically any moist environment. Identification of diatom has application in many disciplines, including ecology, archaeology and forensic science. In recent years, the work has been undertaken for automatic identification of diatom. However, the diatom with circular shape has not yet been considered as the uttermost goal of the research. In this research, a method based on texture feature for automatic identification of circular diatom images is presented. We aimed in this research to find the exact location of diatom by using the image segmentation and segmentation adjustment first, and then obtain eigenvectors by Fourier spectrum features, and finally use a BP neural network to effectively classify the diatoms with circular shape. With classification carried out using a BP neural network we attained 94.4% accuracy from a set of image containing twelve spieces of circular diatom. These species include mainly: *Coscinodiscus oculatus* (Fauv.) Petit, *Coscinodiscus kuetzingii* A. Schmidt, *Coscinodiscus radiatus* Ehrenberg, *Coscinodiscus asteromphalus* var. *asteromphalus* Ehrenberg, *Coscinodiscus excentricus* Ehrenberg, *Coscinodiscus curvatulus* var. *curvatulus* Grunow, *Coscinodiscus wittianus* Pantocsek, *Arachnoidiscus* Bailey, *Cyclotella* Kutzing, *Actinoptyclrus* Ehrenberg, *Actinocyclus* Ehrenberg, *Hyalodiscus* Ehrenberg. The result is an effective attempt of circular diatom identification based on texture character.**

Index Terms—**circular diatom; microscopical image; automatic identification; texture analysis; segmentation adjustment; fourier spectrum; neural network;**

I. INTRODUCTION

Diatoms are a large and ecologically important group of unicellular algae found in almost all aquatic habitats. They are responsible for approximately 40% of marine primary productivity[1] and play a key role in the ocean's silicon cycle[2]. As a crucial source of food for marine life like zooplankton, fish, shellfish, Diatoms are an important link of food chain in aquatic ecosystem. They can be of great significance for the balance of ecosystem. Moreover, diatom has been used in wide range of applications[3], including water quality assessment, climate change, oil exploration, archaeology and forensic science. All of these applications require identification of diatom.

The outside silica cell wall (frustule) surrounding diatom cell, which is composed of pieces of highly patterned and perforated hydrated silica ($SiO_2 \cdot nH_2O$), is a special feature making them easily recognizable. The frustule is constructed of two large elements call valves, which typically overlap one over the other like the two halves of Petri dish. In most diatom species, their valves are flat enough to give a repeatable two-dimensional(2D) view in all photographs. In this research, we extract the external 2D contours of such valves as the shape of diatoms, and texture features of the diatoms[4]. There is a wide variety of the size, shape and texture that frustule can take, and these characteristics are traditionally used by taxonomists to classify diatom species. In practice, it is very difficult for taxonomists to identify huge number of diatom species (approximately 2×10^5[5]) exactly. Therefore, microscope scanning system could be combined with digital image processing and pattern recognition analysis to establish a system of automatic identification of diatoms due to difference and stability in their frustule.

Currently, the methods of automatic identification of algae, including absorption spectroscopy[6], fluorescence spectroscopy[7], liquid chromatography[8], flow cytometry, molecular genetic techniques and so on, are not only tedious but also solely depend on physiological state of algae with low resolution (most of them could divide algae to classes except molecular genetic techniques).

Up to now, few researches on automatic diatom identification has been conducted. In 1998, the Automatic Diatom Identification and Classification (ADIAC)[9] started in order to explore the computer processing of

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diatom images. In this project, contour and texture characteristics of Pennatae diatoms were used for classification and 97% of results from a set of images including 37 diatom species were achieved.

However, diatom morphology (Fig. 1) varies, although, some cells are triangular, square, elliptical and so on, the typical diatom cell shape is circular, especially in marine species[10]. Circular diatoms are an important group of marine phytoplankton community. It is well known that marine diatoms, in their various applications such as red tide forecast, marine ecological investigation and so on, need to be classified.

However, the identification of circular diatoms was not considered as the uttermost goal of the ADIAC project, which was rather the identification of diatoms of other shapes distributed in the British freshwater lake and coastal waters of Northern Europe. The methods suggested in the ADIAC project and other studies are not applicable to our problem, as the texture features of circular diatom are different.

Therefore, for automatic identification of marine diatom, an effective method to deal with the feature selection, feature extraction, and classification of circular diatom texture is to be developed.

II. MATERIALS AND METHODS

A. Image Acquisition

The diatom images used in the work were captured from diatom strains collected from coastal waters of China and kept in the Diatom laboratory of Xiamen University, China.

Diatom samples were analyzed using OLYMPUS BX41 optical microscope, at low magnification (10×), medium magnification (20×), and high magnification (40× and 100×). Image acquisition was performed using OLYMPUS DP50 assisted with computer software Viewfinder Lite V1.0. The original resolution of diatom image was 2776×2074 pixels.
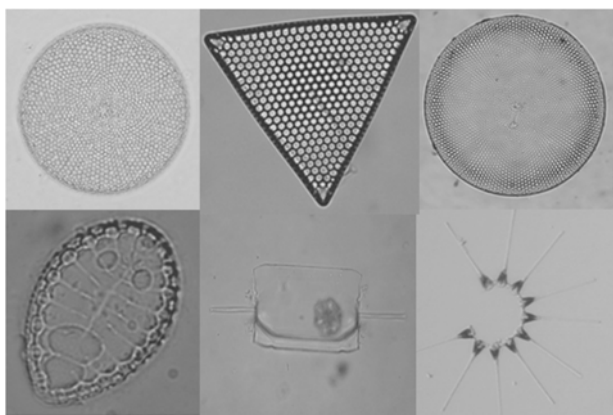
The image database contains six genera of circular

Figure 1. Light microscope images of diatom species

From left column to right column, Line 1: *Coscinodiscus argus* Enhrenberg, *Triceratium favus* Enhrenberg, *Coscinodiscus asteromphalus* Enhrenberg; line 2: *Surirella fluminensis* Grunow, *Ditylum brightwelli* (West) Grunow, *Asterionella japonica* Cleve

diatom (Fig. 2) of which: The Genus *Coscinodiscus* Ehrenberg, Genus *Arachnoidiscus* Bailey, Genus *Cyclotella* Kutzing, Genus *Actinoptyclrus* Ehrenberg, Genus *Actinocyclus* Ehrenberg, Genus *Hyalodiscus* Ehrenberg. The Genus Coscinodiscus Ehrenberg is represented by seven species in this work notably: *Coscinodiscus oculatus* (Fauv.) Petit, *Coscinodiscus kuetzingii* A. Schmidt, *Coscinodiscus radiatus* Ehrenberg, *Coscinodiscus asteromphalus* var. *asteromphalus* Ehrenberg, *Coscinodiscus excentricus* Ehrenberg, *Coscinodiscus curvatulus* var. *curvatulus* Grunow, *Coscinodiscus wittianus* Pantocsek. It is worthy to note that there are not only interspecies differences but also intergeneric differences in these twelve species. For each species, 15 images were selected.

B. Operating Platform and methods

The operating platform used involves AMD Athlon 64 CPU, 80GB HD, 1GB RAM, Windows XP professional and so on. Image processing and pattern recognition were performed using computer software MATLAB 7.0 Circular diatom identification appeared to be mainly dependent on the classification of texture feature. However, in practice, for accurate texture identification, the first thing needed is exact texture image, which is an accurate segmentation result.

Unfortunately, it is difficult to detect a perfect round contour in the initial segmentation process because of some effects. In this regard, a valve may not be level such that part of its contour is not well focused, or also dust specks caused by particles in another focal plane and therefore blurred, in addition, debris or a broken valve touches the contour of a potentially usable valve and so on. All these factors may significantly affect the following procedure of segmentation. In light of the mentioned problems a segmentation adjustment of circular diatom is required.

Based on the optical characteristics of the circular diatoms observed under microscope, the complete method consists of several steps:

1) Image segmentation

2) Find the exact location of diatom by segmentation adjustment

3) Obtain eigenvectors by Fourier spectrum features

4) Classify the diatoms with circular shape using a BP neural network

III. THE IMAGE SEGMENTATION

Image segmentation is one of the most important tasks in image processing. All the colour images captured by microscope are converted into grey level images before computations using the standard conversion, as in (1), where R, G, and B represent the red, green and blue colour channels, respectively [11].

$$Y = 0.2989 \cdot R + 0.5870 \cdot G + 0.1140 \cdot B \qquad (1)$$

Although point and line detection certainly are important in any discussion on image segmentation, edge detection is by far the most common approach for detecting meaningful discontinuities in intensity values,
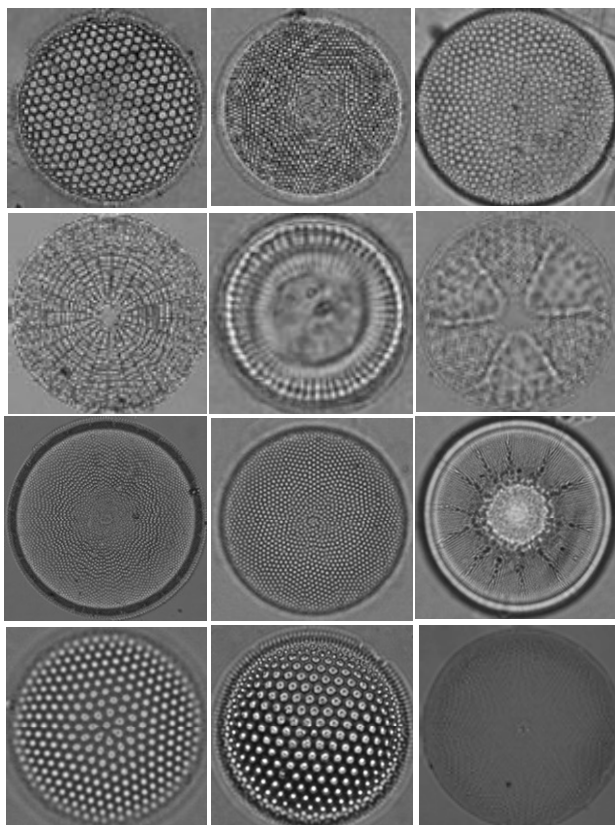
Figure 2. The sample set of circular diatom

From left column to right column, Line 1: *Coscinodiscus oculatus* (Fauv.) Petit, *Coscinodiscus kuetzingii* A. Schmidt, *Coscinodiscus radiatus* Ehrenberg; Line 2: *Arachnoidiscus* Bailey, *Cyclotella* Kutzing, *Actinoptyclrus* Ehrenberg; Line 3: *Actinocyclus ehrenbergii* var. *ehrenbergii* Ralfs, *Coscinodiscus curvatulus* var. *curvatulus* Grunow, *Hyalodiscus* Ehrenberg; Line 4: *Coscinodiscus excentricus* Ehrenberg, *Coscinodiscus wittianus* Pantocsek, *Coscinodiscus asteromphalus* var. *asteromphalus* Ehrenberg.

which is called the edge of image. Therefore in the work we used the Canny edge detector[12] which is the most powerful edge detector provided to segment image. Canny edge detector is an approach to find places where the first derivative of the intensity is greater in magnitude than a special threshold, and it can be summarized as follow[13]:

A. *The image is smoothed using a Gaussian filter with a specified standard deviation, σ, to reduce noise.*
B. *The local gradient (2) , and edge direction (3), , are computed at each point. G_x and G_y can be computed by first deviative of the intensity. An edge point is defined to be a point whose strength is locally maximum in the direction of the gradient.*

$$g(x, y) = [G_x^2 + G_y^2]^{1/2} \qquad (2)$$

$$\alpha(x, y) = \tan^{-1}(G_x^2 + G_y^2) \qquad (3)$$

C. *Then the nonmaximal suppression in the gradient magnitude image to give a thin line, which is the ridges of the edge points determined in (B). The*

ridge pixels are then thresholded by two thresholds, τ_1 and τ_2, with $\tau_1 < \tau_2$. Ridge pixel with values greater than τ_2 (the default value is 0.7-0.8 in matlab) are said to be "strong" edge pixels, and ridge pixel with values between τ_1 (in matlab the default value is $\tau_1 = 0.4\tau_2$) and τ_2 are said to be "weak" edge pixels.

D. *Finally, the algorithm performs edge linking by incorporating the weak pixels that are 8-connected to the strong pixels.*

Consequently, the threshold value of Canny edge detector has a great influence on result, and the value of σ was also important. Using the default threshold value ($\tau_1 = 0.4\tau_2$, $\tau_2 = 0.7$) in matlab, the poor result was found. So after repeated experiments, the result was consistent with the values of $\tau_1 = 0.003, \tau_2 = 0.012$, and $\sigma = 1$.

IV. SEGMENTATION ADJUSTMENT

Nevertheless, if the diatom is not properly focused, or if the illumination around a diatom is not uniform, then edges can only be partially detected or large areas of the surrounding background would also be detected. As shown in Fig. 3(Line 2), the result of detecting a perfect round contour would be problematic. In such cases, it may affect the subsequent procedure of segmentation. Thus, the segmentation adjustment is of great significance for circular diatom identification.

Segmentation adjustment requires some prior knowledge of geometric features. It is in that regard that Hough transform[14] is indeed well known approach in detection of round contour of images.

However, the practical problems with this approach are the large computation-memory requirement and parameter extraction limited by quantification of the parameter space. One way to surmount these problems is to decrease the detectable pixels and to narrow the parameter space. In the preliminary experiment, the radius was first estimated using the result from segmentation and then the edge lines segmented were used as the detectable pixels. As a result, the computing time, which is more than ten seconds per image, is too long to fulfill the requirement of field detection. It is in that view that a simple and accurate approach for segmentation adjustment is necessary.

Note that the curve fitting of circle equation is a feasible approach. Furthermore, least square method is usually used in the curve fitting. As Fig. 3 (Line 3) shows, although the method can extract the contour of circular diatoms in the approximate location, it cannot exactly position the contour in the most cases. The reason is that the least quadric error norm was applied to the method of full regression. On the one hand, if the error correlates with normal distribution, then error norm is optimal. In such case, the method is not suitable for that some spots outside the edge. On the other hand, if the

error does not correlate with normal distribution, as a result of existence of a lot of spots outside the edge, then least square method is not an optimal method of curve fitting. In practice, one abnormal valve is probably enough to give a false regression curve. Thus, in order to reduce the impact of abnormal valve，robust regression is advisable.

In the present work, we designed an improved least square method[15] in order to solve the problem presented above. The procedure for this method is as follows. Firstly, the initial contour was divided into several parts, and then the circle equation fitting method was used respectively. The result was consistent with the accuracy of the fit. Moreover, Robust regression method was also attempted to solve the problem. The Robust regression method shown in Fig. 3(Line 4) was found to be more effective than the least square method as shown in Fig. 3(Line 5). Finally, Robust regression method was used for segmentation adjustment of circular diatom.

After segmentation adjustment, that is, achieving precise positioning of circular diatom, we needed to consider that which region of circular diatom should be used for characteristic analyze and characteristic extraction. Therefore, in such case, some thinking strategies were suggested as follow:

A. *Choose the whole region of diatom cell.*
B. *Choose the rectangular region inside the circular diatom contour.*
C. *Choose the circular region with fixed size inside the circular diatom contour.*

As we all know, a lot of characteristics extraction methods require circular symmetry, i.e. radon transform, and the diatom size change in different growth period, so the circular region with fixed size inside the circular diatom contour was used as the area of characteristic analysis. Moreover, zero boundary was used to reduce boundary effect, which is caused by discontinuity of image edge, when using Fourier transform. After setting radius, the grayscale value of region outside the circle was set to zero by Bresenham's circle algorithm.

V. THE TEXTURE FEATURE EXTRACTION

Texture is an important character of diatom and texture extraction is one of the main subjects in pattern recognition. The methods[16] of texture feature extraction include mainly: radon transform, autocorrelation function, gray level co-occurrence matrices, Fourier spectrum and so on. Radon transform could represent the texture structure of an object, especially the changes of texture structure, which varied with circumferential angle within circular object. Autocorrelation function represents definite periodicity which is the distance of neighboring texture primitive for images with repeated texture pattern. This function decreased slowly with coarse texture, and contrarily quickly with fine texture. Therefore, it was necessary to detect texture periodicity and size of texture primitive.
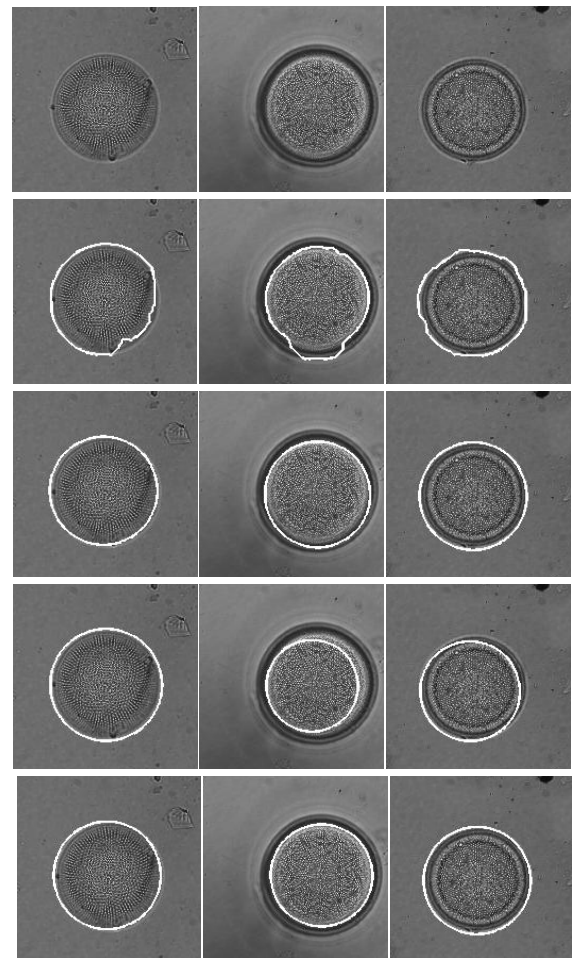

Figure 3 Segmentation adjustment on the circular diatom Results are shown with white line, left column: *Coscinodiscus jonesianus* (Grev.) Ostenfeld; middle, right column: *Actinocyclus ehrenbergii* var. *ehrenbergii* Ralfs. Line 1: original image; line 2: initial segmentation; line 3: least square method; line 4: improve least square; line 5: Robust regression method.

Gray level co-occurrence matrices[17] is based on statistically sampling in such way that certain gray levels occur in relation to other gray levels. This means that spatial relations of texture properties are estimated by using second-order statistics. This method is very suitable for describing microtexture, and unsuitable for texture with big area primitive because matrices didn't contain structural information, which made up a large proportion of texture differences between circular diatoms. So Consequently Gray level co-occurrence matrices are unfit for describing texture. Similarly, because of big differences between texture types of diatom and different texture primitive, autocorrelation function has limited capacity to describe texture feature. Therefore, we used Fourier spectrum to describe texture feature of diatom.

Fourier spectrum is ideally suitable for describing the directionality of periodic or almost periodic two-dimensional patterns in a round image. Image characteristics are quite easy to detect with spatial methods, the size of texture primitive and spatial organization detection as well. In order to describe
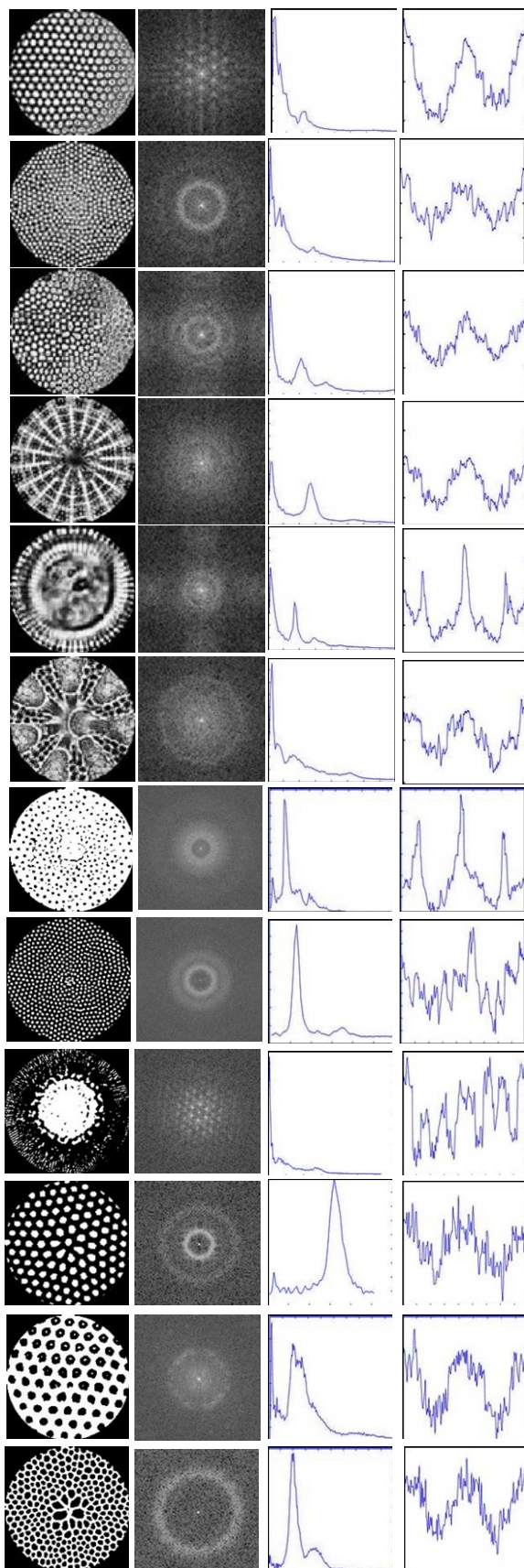
Figure 4. Fourier spectrum features of diatom images

Column 1: Original image; Column 2: Fourier spectrum; Column 3: The plots of radius function; Column 4: The plots of angle function. From Line 1 to Line 6: Coscinodiscus oculatus (Fauv.) Petit, Coscinodiscus kuetzingii A. Schmidt, Coscinodiscus radiatus Ehrenberg, Arachnoidiscus ehrenbergii var. ehrenbergii Bailey, Cyclotella Kutzing, Actinoptyclrus Ehrenberg, Actinocyclus ehrenbergii var. ehrenbergii Ralfs, Coscinodiscus curvatulus var. curvatulus Grunow, Hyalodiscus Ehrenberg, Coscinodiscus excentricus Ehrenberg, Coscinodiscus wittianus Pantocsek, Coscinodiscus asteromphalus var. asteromphalus Ehrenberg.

spectrum features, we could express the spectrum in polar coordinates to yield a function $S(r,\theta)$. Radius function($P_1(r)$) and angle function($P_2(\varphi)$) obtained by annularity sampling of the function $S(r,\theta)$ are one-dimension functions. Radius function, $P_1(r)$, reveals energy distribution information with different frequency. In the condition of coarse texture, if the value of r is smaller, the value of function $P_1(r)$ is small. Nevertheless, in the microtexture condition, the changes of r value has no significant impact on the value of function $P_1(r)$. When the lines and edges of texture image abound in the direction θ, the plots of function $P_2(\varphi)$ in Fig. 4(Column 4) show peaks near (4). If the texture image lacks characteristic direction, the spectrum has no direction neither.

$$\varphi = \theta + \frac{\pi}{2}$$

(4)

VI. NEURAL NETWORKS

Interest in neural networks dates back to the early 1940s, which has spread though many fields, including information processing, pattern recognition, intelligent control, and so on. The performance of most decision-theoretic classifier depend on the accuracy of the actual pattern populations in satisfying the underlying statistical assumptions, while the statistical properties of the pattern classes in this problem are unknown and thus cannot be estimated. The neural network was selected as texture feature vector classifier because it adaptively develop the coefficients of decision functions directly via training. The main advantage of using ANN is its ability to learn a complex nonlinear relationship with limited prior knowledge of the object.

Minsky and Papert showed that a multilayer feed-forward network can counteract many restrictions associated with single-layer network. However, the computational effort needed for finding the correct combination of weights increases substantially when more parameters and more complicated topologies are considered. The back-propagation algorithm provides an effective training method for multilayer machines. The central idea behind this solution is that the errors for the units of the hidden layer are determined by back-propagating the errors of the units of the output layer. Multilayer perceptron neural network employing back propagation training algorithm has been used successfully in numerous problems of practical interest.

Considering that Principal Component Analysis (PCA) [18]is a powerful multivariate data analysis method. Its

main purpose in sense that it reduces and summarizes large and high dimensional datasets by removing redundancies. It is a useful statistical technique that has found many applications in different scientific fields. We perform PCA on the texture feature datasets obtained using Fourier spectrum method.

Four combinations of feature patterns are further reduced respectively.

A. Only $P_1(r)$ Fourier frequency spectrum radius function feature vector，the vector length is reduced from 40 to 20;

B. Only uses $P_2(\varphi)$ Fourier frequency spectrum angle function feature vector, the vector length is reduced from 90 to 30;

C. Both $P_1(r)$, $P_2(\varphi)$, the total vector length is reduced to 50;

D. Both $P_1(r)$, $P_2(\varphi)$, only $P_2(\varphi)$ is compressed to the length of 10.

All the reduced dataset is given as input to the neural network for classification. Several Neural Networks are designed with 15，30，40，50，60，80 nodes in the single hidden layer and 6 nodes for each class in the output layer to test the performance using leave one out methods.

$P_2(\varphi)$ feature vectors did not give satisfactory results. We suspect that reflecting radial symmetrical texture pattern is not sufficiently discriminating, because most circular diatom considered here have the similar symmetrical texture pattern. It seems that the radius function $P_1(r)$ represent a discriminative structural texture information, which can be successfully used in diatom identification. However, the most promising result 94.44% was reached by combining these two feature pattern(Table I).

VII. CONCLUSIONS

In this work, we designed an automatical identification method of circular diatom. In this approach, First, we get the edge map of microscopic image using Canny detector, Second an improved least square method which pinpointed the circular texture region was presented. Third, we extracted the radius function $P_1(r)$ and, the angle function $P_2(\varphi)$ of Fourier spectrum, and analyzed the PCA compression dimension for the principal components of $P_1(r)$ and $P_2(\varphi)$. Finally, we used BP neural networks to carry out classification using discriminant. In the case of small samples (15 images per species), we obtain a promising result with 94.44% from twelve spieces of circular diatom as individual expert's identification rates ranged from 43% to 86.5%[19], this method can compete with well-trained experts. Based on results from this research, circular diatom identification using microscopic images approach is potentially applicable in future automatic identification of microalgae in the field of phycology.

TABLE I.
CLASSIFICATION RATE FROM DIFFERENT FEATURE PATTERN COMBINATIONS AND THE NEURON NUMBERS IN HIDDEN LAYER

Feature Pattern	The Original Length of Feature Pattern	The Length of Feature Pattern after PCA	Neuron Numbers in Hidden Layer	Classification Rate（%）
$P_1(r)$	40	20	15	89.44
			30	90.56
			50	88.33
$P_2(\varphi)$	90	30	30	60.56
			40	58.33
			50	58.89
$P_1(r)$, $P_2(\varphi)$	130	20+30	40	69.44
			60	71.67
			80	76.67
$P_1(r)$, $P_2(\varphi)$	130	40+10	40	88.89
			60	90.56
			80	94.44

$P_1(r)$ is the Fourier frequency spectrum radius function, $P_2(\varphi)$ is the Fourier frequency spectrum angle function

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