RESEARCH ARTICLE

Annular characteristic spectrum extraction for species identification of marine *Coscinodiscus* from micrographs

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Phytoplankton represented by *Coscinodiscus* plays a very important role in maintaining the global marine ecological balance. How to identify the dominant algae species effectively and accurately has become a difficult problem for researchers to solve. In this study, combined with the biomorphological characteristics and classification knowledge of phytoplankton, we design and apply a variety of technologies of image analysis and computer vision to make an in-depth research on the problem of automatic cell discrimination and species identification of marine *Coscinodiscus* from micrographs. A novel *Coscinodiscus* species identification strategy based on annular characteristic spectrum extraction was put forward. In the cell extraction stage, the maximum discrete measure matrix trace (MDMMT) method and some appropriate morphological processing were carried out to extract the cell targets from the grayscale image. In the *Coscinodiscus* discrimination stage, the circularity measurement and Hough circular target detection for the unknown connected regions in the binary images were executed successively to distinguish *Coscinodiscus* cells from a large number of phytoplankton binary images. In the feature extraction stage, the inscribed circle of each *Coscinodiscus* cell target was divided into four annular regions, and the equivalent pattern local binary pattern (LBP) texture characteristic spectrums with rotation invariance of these four annular regions were extracted, respectively as classification basis, which were then converted into normalized statistical histograms one by one. Since the dimension of each histogram was 59, the total feature dimension was 59 × 4 = 236. Support vector machine (SVM) was adopted as classifier to perform pattern recognition on the LBP texture feature data. The results demonstrated that the proposed strategy based on annular characteristic spectrum extraction achieved an average recognition accuracy of up to 85.98%, which could effectively and accurately realize the species recognition of common marine *Coscinodiscus*.

Keywords: cell extraction; Coscinodiscus discrimination; circularity measurement; circular target detection; annular characteristic spectrum; species identification.

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Introduction

The observation of marine phytoplankton community structure and its diversity has great scientific and practical significance for guiding fishery production, monitoring the marine environment, ecosystem health assessment, and ecological disaster emergency monitoring (especially red tide warning) [1]. The focus of phytoplankton monitoring is to investigate its species composition, quantity distribution, and temporal and spatial changes. The most important monitoring indicator is the species of dominant algae. *Coscinodiscus* is a general term

for a large diatom class of planktonic microalgae, belonging to *Bacillariophyta*, *Centricae*, *Discoidales*, and *Coscinodiscaceae* [2-4]. According to statistics, this genus has a total of about 450 species worldwide including freshwater, seawater, and fossilized species with the most of them are marine phytoplankton. There are more than 51 species of *Coscinodiscus* in China. Traditional phytoplankton identification mainly relies on experienced algologist to manually observe the morphology under an optical microscope or even an electron microscope to determine the specific species, which is universally acknowledged as the most intuitive and accurate classification method. However, it requires a high level of professional knowledge and rich experience in classification and needs the classification staff to work meticulously for a long time.

In recent years, computer vision technology-based phytoplankton microscopic image recognition method has increasingly attracted the attention of academia because it has the ability to automatically classify to the "species" level, which is the basic unit of biological classification. The super image information processing capability possessed by the computer combined with the microalgae's biomorphological structural characteristics has laid a solid foundation for the rapid and automatic analysis of micrographs. Sosik and Olson designed a feature selection method that integrated a series of image features including shape, size, orientation invariant moments, and co-occurrence matrix statistics, which was used for 22-category classification by Support Vector Machine (SVM) [5]. Tao *et al*. designed a real-time SVM marine red tide algae classifier for flow-cytometry algae monitoring system that could use Support Vector Data Description (SVDD) to reject non-target algae and contaminative targets [6]. Dimitrovski *et al*. proposed a hierarchical multi-label classification strategy for diatom classification by building Predictive Clustering Trees (PCTs) that could simultaneously predict different taxonomic levels of genus, species, and form [7]. Verikas *et al*. put forward an image processing technique to detect and identify *Prorocentrum* minimum species in light and fluorescent microscopic images of phytoplankton, which selectively extracted several features characterizing cell contours and then utilized SVM and Random Forest (RF) as classifiers to distinguish *Prorocentrum* minimum cell from other objects [8]. Ouyang *et al*. designed a deep convolutional neural network model for automated plankton image classification which applied rotational and translational symmetry [9]. Lee *et al*. proposed a fine-grained plankton classification strategy for large-scale database by using Convolutional Neural Network (CNN) and incorporated transfer learning to solve the class-imbalance problem through pre-training CNN with class-normalized data and fine-tuning with original data [10]. Dai *et al*. developed a CNN-based automatic plankton classification system by extracting global and local features to describe shape and texture information of plankton, and specially designed a fully connected pyramid network structure to merge inner products from different sub networks [11]. Zheng *et al*. proposed an image classification algorithm for plankton that depended on multi-view features obtained by multiple kernel learning (MKL) [12]. Li *et al*. introduced a machine learning technique based on Mueller matrix imaging system to distinguish eight species of morphologically similar microalgae and one species of cyanobacteria through CNN [13], while Liu *et al*. proposed a deep learning method based on Deep Pyramidal Residual Networks (PyramidNet) for plankton image automatic classification [14]. Giraldo-Zuluaga *et al*. proposed an automatic identification methodology with SVM and Artificial Neural Network (ANN) for digital microscopic images of *Scenedesmus* microalgae, in which they used adaptive contrast histogram equalization for pre-processing, active contour for segmentation, and statistical features for algal characterization [15]. Deglint *et al*. extracted fluorescence-based spectral-morphological features and used machine learning to automatically recognize and enumerate six types of microalgae [16], and, in

addition, designed an automatic classification system by using a deep residual CNN to recognize six types of microalgae by integrating morphological features and their corresponding multi-wavelength signals [17]. Lumini and Nanni presented a pre-trained CNN structure for plankton automated recognition based on the fusion of several deep learning models, focusing on the fine tuning of different models, and transferring learning of the same model [18]. Park *et al*. used a neural architecture search (NAS) approach in ANN to find the best CNN model for the automatic classification of eight algae genera in watersheds experiencing algal blooms, including three diatoms, three cyanobacteria, and two green algae [19]. Furthermore, Park *et al*. proposed a hierarchical learning method by using semantic feature of Nonnegative Matrix Factorization (NMF) for the harmful red tide algal image automatic identification [20]. Qian *et al*. put forward a novel end-to-end multi-target deep learning strategy for algal detection and biological class/genus classification and achieved a colored microscopic algae dataset containing 27 genera [21]. Liu *et al*. applied a kind of CNN to classified 12 species of marine microalgae from 5 families through low-resolution Mueller matrix images that were captured by Mueller matrix microscopy with LED light source at 514 nm wavelength [22].

According to previous studies, whether it is machine learning or deep learning based on CNN, there are few studies on target detection and species identification, specifically for the *Coscinodiscus* micrographs. Although deep learning technology has been developed rapidly and demonstrated great achievements in image analysis in the past five years, building deep learning models still requires training in large instance-level labeled image datasets. The lengthy and cumbersome data annotation obviously hinders the development of deep learning algorithms in the detection and classification of phytoplankton micrographs with complex biomorphological details. This study designed and applied a series of technologies of image analysis and computer vision to solve the problem of automatic cell discrimination and species identification of *Coscinodiscus* by combining the classification knowledge and biomorphological characteristics of marine phytoplankton in micrographs. This research would provide the possibility of on-site and *in situ* automatic classification for large batches of *Coscinodiscus* sample images.

Materials and methods

MATLAB R2019B (64-bit) software (MathWorks, Natick, MA, USA) was employed for the programming of this study under the Windows 10 operating system (Microsoft, Redmond, WA, USA).

Specimen image collection

A total of 10 Coscinodiscus species including *Coscinodiscus curvatulus*, *Coscinodiscus subtilis*, *Coscinodiscus wailesii*, *Coscinodiscus argus*, *Coscinodiscus radiatus*, *Coscinodiscus oculus-iridis*, *Coscinodiscus asteromphalus*, *Coscinodiscus apiculatus*, *Coscinodiscus nodulifer*, and *Coscinodiscus excentricus* were involved in this study based on the analysis of the population structure, distribution, and occurrence frequency of planktonic microalgae in the coastal waters of China including the Bohai Sea, the Yellow Sea, the East China Sea, and the South China Sea. All samples were collected from the South China Sea and the East China Sea, and their optical micrographs were captured by using Olympus BX optical microscopes (Olympus Corporation, Tokyo, Japan) with high-speed micrography system QImaging Retiga 4000R FAST 1394 CCD (QImaging Corporation, Surrey, British Columbia, Canada).

Cell extraction

A multicellular target extraction strategy was designed in this study by using Maximum Discrete Measure Matrix Trace (MDMMT) method (Figure 1). The input color micrograph was first converted into a grayscale image to remove the hue and saturation information and retain only the brightness. The gray values of the

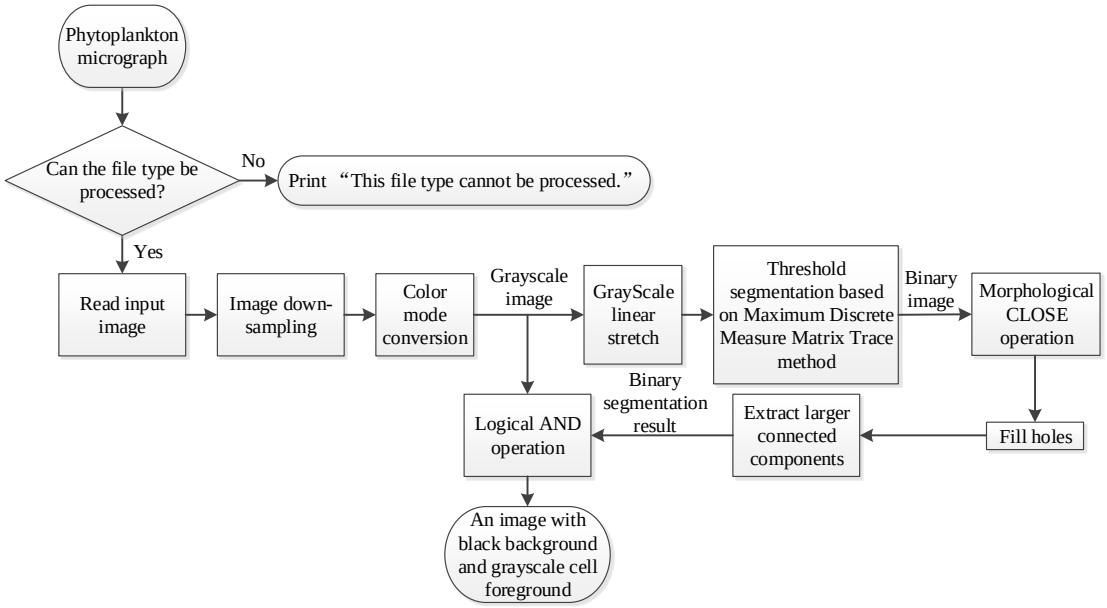

Figure 1. Flow chart of multicell extraction by using Maximum Discrete Measure Matrix Trace (MDMMT) method.

grayscale images were then linearly mapped to enhance the contrast. After gray stretching, the new image became brighter and owned a more prominent cell region. Given the vast grayscale difference between the cellular target and the background, MDMMT algorithm was applied for threshold segmentation. A 2D gray histogram was built and used to segment the stretched grayscale image. For the acquired binary image, morphological CLOSE and hole filling operation were performed to repair broken contours, remove noise, and reconnect several adjacent regions that might be broken into pieces by mistake. Considering that there might be multiple cells in one image, further extraction of those connected components with larger areas was performed. Since the biggest connected region was usually an algae cell, those connected regions whose areas were equal to or larger than 35% of the biggest connected region's area were extracted. At the end of the process, the generated binary image and the initial gray image were logically AND operated and merged into one image with gray-level cellular foreground and black background as the final extracted result.

Coscinodiscus discrimination

When many cells were extracted in batches, it was necessary to find a machine discrimination method to automatically judge whether they belonged to *Coscinodiscus*. The external shape characteristics of a cell were parameterized and mathematically defined and quantified the degree of its proximity to the circle (circularity). A reasonable circularity threshold through a large number of experimental data was manually selected with the circularity of a cell greater than the threshold being preliminarily determined as *Coscinodiscus*. Hough Transform was then used to detect circular targets in the image of suspected *Coscinodiscus* cells before the final determination of *Coscinodiscus* cells and the cell number by computer.

(1) Circularity measurement

The connected regions in binary segmented cell micrographs were labeled to calculate the Mark Matrix. Specifically, assuming that there was *n* connected regions (numbered 1, 2, …, *n*, respectively), all pixels in each connected region were assigned the same number. Then a Mark Matrix consistent with the size of the binary image was created and the numbers at the

corresponding positions in the Mark Matrix were recorded. Therefore, all elements with a value equal to k in the Mark Matrix represented the pixels of the k-th ($1 \le k \le n$) connected region. The contour perimeter C_k of k-th connected region was calculated according to the coordinates of its boundary pixels. The descriptors of k-th connected region could be obtained through the Mark Matrix including the area S_k, centroid coordinates, and bounding box. The circularity of the k-th connected region was defined as follows.

$$R_k = \frac{4\pi \cdot S_k}{C_k^2}, \ 1 \le k \le n \quad (1)$$

By comparing a large amount of experimental data on circularity measurement, 0.65 was selected as the circularity threshold as the preliminary basis for judging *Coscinodiscus* because there were obvious differences in the circularity of cells between *Coscinodiscus* and non-circular phytoplankton. When the circularity of a connected region was greater than the threshold 0.65, it could be preliminarily determined as a *Coscinodiscus* cell. In this case, its regional centroid could be approximately regarded as the center of the circle and marked with a red dot. Then, the Region of Interest (ROI) on image defined by the bounding box was cropped from its corresponding image with black background and gray cell foreground. If no circular target was detected in a Mark Matrix, the original micrograph corresponding to this matrix was automatically classified as a non-*Coscinodiscus* image.

(2) Circular target detection

For the microscopic image that existed *Coscinodiscus* cells preliminarily determined in the previous step, it needed to be further verified by Hough Transform detection. Hough Circle Transform is a fast and effective technique for detecting circles in images [23]. In order to reduce the number of noise and the risk of false detection, Gaussian low-pass filter was employed to linearly smooth the preliminarily determined *Coscinodiscus* gray image, and then the filtered image was used as the input image of Hough Circle Transform algorithm. All parameters were set to appropriate fixed values or automatically obtained values for image batch detection. For the suspected *Coscinodiscus* cell screened through the circularity measurement link, if no circular target was detected through the Hough Transform link, it would be recognized as non-circular phytoplankton. Otherwise, it could be finally recognized as *Coscinodiscus*.

Annular characteristic spectrum extraction

The equivalent pattern LBP texture descriptor with rotation invariance was used to construct a 59-dimensional feature vector. The original algorithm was improved to extract only the characteristic spectrums of four annular regions inside one *Coscinodiscus* cell. Since the lucolus on the shell surface of *Coscinodiscus* is arranged radially or spirally from the center to the shell edge, the circular cell targets were divided into four annular regions and their characteristic spectrums were extracted respectively. Because the edge of the connected region was not neat enough, it might affect the symmetry of the pattern. Therefore, the inscribed circle of the connected region was first used to replace the original connected region. Then the inscribed circle was cut into 1/4, 2/4, 3/4, and a whole (4/4) circle. By making a difference between adjacent sub circles, four annular regions could be obtained and were named as Blocks 1, 2, 3, and 4, respectively. The ROI of each block was standardized and placed on black square masks. Local Binary Pattern (LBP) is an operator used to describe the local texture features of an image [24]. Assuming that the number of sampling points in the neighborhood is n, the number of traditional LBP patterns can reach 2^n. An improved equivalent pattern LBP algorithm with rotation invariance was used to reduce the types of patterns [25], which made the number of patterns sharply reduced to $n \times (n - 1) + 2$ equivalent patterns and 1 mixed pattern. For the 3×3 neighborhood with 8 sampling points, the number of patterns was reduced from the initial 256 to only 59. Among them, 58 equivalent pattern types could contain most of the original

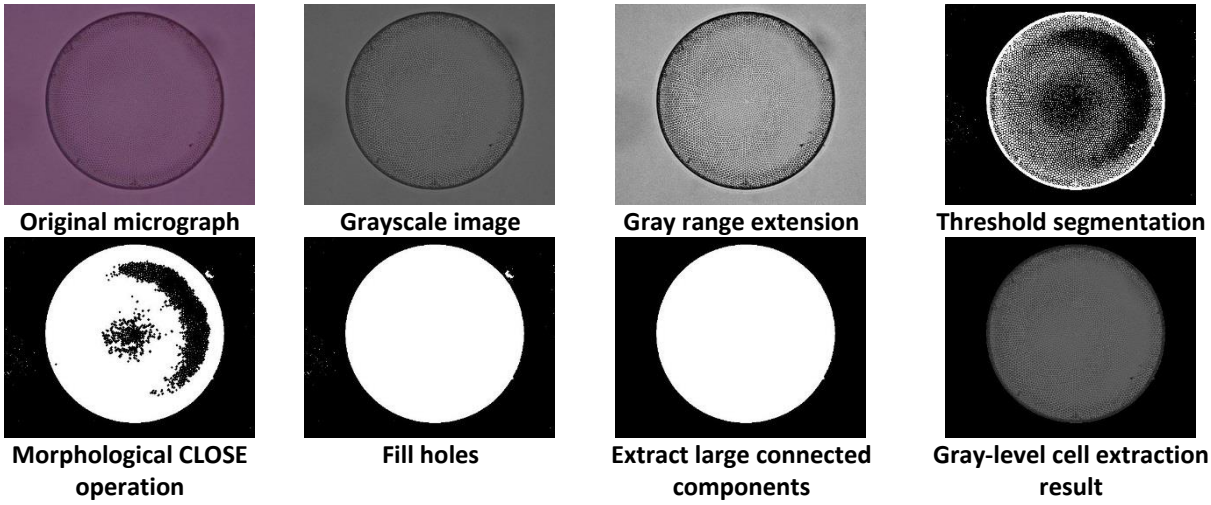

Figure 2. *Coscinodiscus oculus-iridis* cell extraction pipeline.

LBP values, while the remaining LBP values were included in the 59th mixed pattern type. Through the equivalent pattern LBP algorithm, each pixel in a gray image could be assigned an LBP code. The LBP codes of all pixels could be extracted to construct a new image, which was called LBP characteristic spectrum. The characteristic spectrum was then transformed into the form of statistical histogram to obtain a 59-dimensional feature vector. Considering that, when the number of pixels in each region was different, the components in the corresponding feature vector were not consistent. The total number of pixels in each annular region was used to normalize their histograms, respectively.

Results and discussion

A total of 500 microalgae micrographs were tested to discriminate *Coscinodiscus* from other species including 350 non-circular phytoplankton micrographs and 150 *Coscinodiscus* micrographs. The results showed that only 3 *Coscinodiscus* images were wrongly identified as non-circular phytoplankton images with two of them due to the connection of *Coscinodiscus* cells with a large area of flocs resulting in shape “distortion” and the circularity measurement value lower than 0.65. In the third image, the edge structures were seriously missing, so no circular target was detected in the Hough Transform stage. The total discrimination accuracy of *Coscinodiscus* micrographs was 99.4%.

All the process images of cell extraction exemplified by *Coscinodiscus oculus-iridis* were displayed in Figure 2. The results showed that our extraction strategy could not only effectively remove various types of noise, but also retain the complete shape of *Coscinodiscus* cell as much as possible. An intuitive visualization technology was applied to mark the circularity. Each Mark Matrix was converted into a true color image for display, in which the background and connected regions were filled with different colors for differentiation (Figure 3). The contour and center of the detected circle on the microscopic image were drawn. The results showed that Gaussian filter made the gray image blurred, and significantly weakened the drastic change of gray at the cell edge, making it a smooth transition. The detected circle had the center and radius values close to the minimum surrounding circle. On the other words, if two circles appeared in the same image, they were similar in size, not far apart in position, and some contours would even coincide, which manifested that the algorithm had excellent detection effect for circular targets (Figure 4).

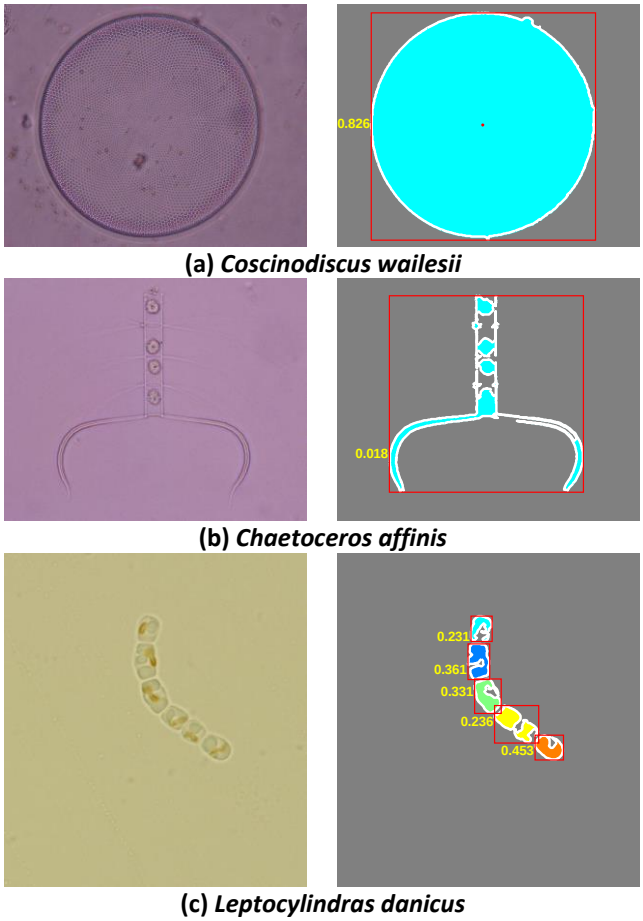

Figure 3. Circularity measurement.

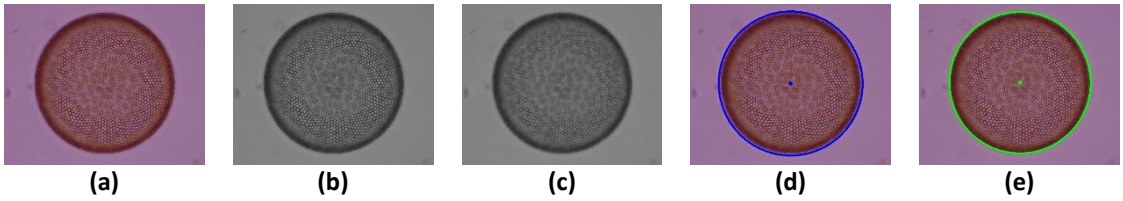

Figure 4. Circular target detection process in *Coscinodiscus argus* image (500×400). **(a)** original micrograph; **(b)** grayscale image; **(c)** Gaussian filtering; **(d)** the circumscribed circle of a connected region with a center of (250, 197) and a radius of 180; **(e)** the detected circle with a center of (251, 195) and a radius of 176.

Taking *Coscinodiscus asteromphalus* cell as an example, the processing process of inscribed circle, sub circles, and annular regions was shown in Figure 5(a)-(d). The LBP characteristic spectrums and their corresponding normalized statistical histograms of the four annular regions of a *Coscinodiscus asteromphalus* cell were shown in Figure 5(e) and Figure 6.

SVM based on radial basis kernel function was used as classifier to train and recognize LBP texture feature data. In this study, a total of 600 *Coscinodiscus* micrographs were selected including 400 micrographs for training and 200 micrographs for recognition. The results showed that the recognition accuracy of 10 species of *Coscinodiscus* ranged from 77.78 to 94.12% with

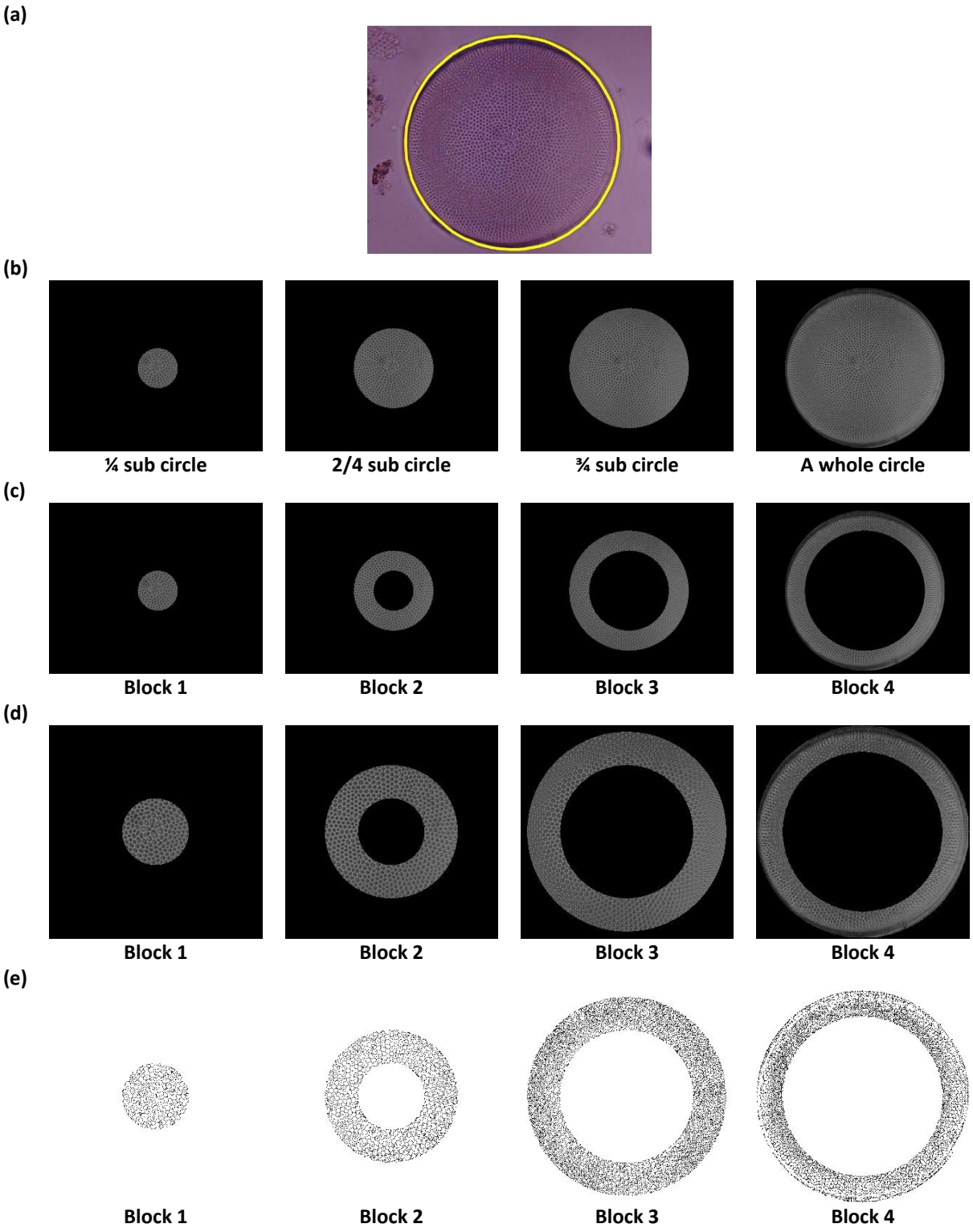

Figure 5. Extraction process of LBP characteristic spectrums of a *Coscinodiscus asteromphalus* cell. **(a)** The inscribed circle of the connected region. **(b)** Cut the inscribed circle. **(c)** Four annular regions of the inscribed circle. **(d)** Standardized square images of four annular regions. **(e)** LBP characteristic spectrums of the four annular regions.

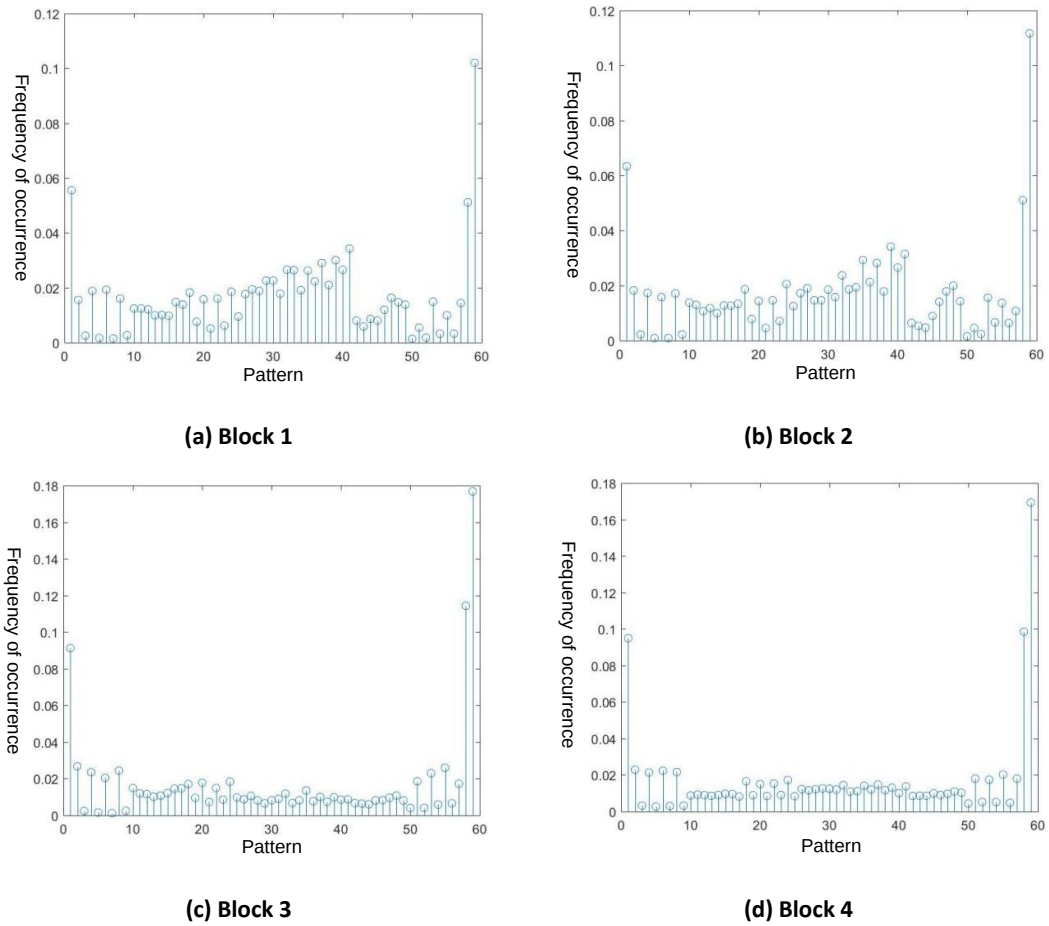

Figure 6. The normalized LBP statistical histograms of the four annular regions of a *Coscinodiscus asteromphalus* cell. (**Note:** the horizontal axis was the sequence number of the pattern, and the vertical axis was the proportion of pixels belonging to a certain pattern to the total number of pixels).

the average recognition accuracy of 86.50%. Due to a certain error rate in the *Coscinodiscus* discrimination stage, the actual recognition accuracy was between 77.31 and 93.55% with the actual average recognition accuracy of 85.98% (Table 1). These data clearly demonstrated that the species identification method of *Coscinodiscus* based on annular characteristic spectrum extraction had achieved encouraging performance.

This study combined knowledge of phytoplankton classification and designed and applied a variety of technologies of image analysis and computer vision to explore a relatively complete species identification scheme for common marine *Coscinodiscus*. The study used biomorphological characteristics of marine phytoplankton in micrographs to explore the problem of automatic cell discrimination and species identification of *Coscinodiscus* by extracting phytoplankton cell targets, determining threshold segmentation using MDMMT method and processing morphological images. *Coscinodiscus* cells were automatically distinguished from a large number of phytoplankton foreground binary images. The circularity measurement and Hough circular target detection for the unknown connected region were performed to judge whether it was *Coscinodiscus* or non-*Coscinodiscus*. In the feature extraction stage, we chose the texture

Table 1. Identification of *Coscinodiscus* species (discrimination accuracy R1 = 99.4%).

Species name	No. of test samples	No. of correctly identified samples	Recognition accuracy R_2	Actual recognition accuracy $R_1 \times R_2$
Coscinodiscus curvatulus	21	18	85.71%	85.20%
Coscinodiscus subtilis	18	14	77.78%	77.31%
Coscinodiscus wailesii	20	18	90.00%	89.46%
Coscinodiscus argus	19	17	89.47%	88.94%
Coscinodiscus radiatus	23	20	86.96%	86.43%
Coscinodiscus oculus-iridis	22	18	81.82%	81.33%
Coscinodiscus asteromphalus	16	14	87.50%	86.98%
Coscinodiscus apiculatus	20	16	80.00%	79.52%
Coscinodiscus nodulifer	17	16	94.12%	93.55%
Coscinodiscus excentricus	24	22	91.67%	91.12%
Overall	200	173	86.50%	85.98%

feature as a classification basis and extracted the rotation-invariant LBP texture descriptor of equivalent pattern. Each circular cell target was divided into four annular regions and the characteristic spectrums of these four annular regions were extracted respectively. Each characteristic spectrum was then converted into the form of normalized LBP statistical histogram representing a 59-dimensional feature vector with the total feature dimension of 236. SVM was then used as classifier to perform pattern recognition on the LBP texture feature data to realize specific species identification of *Coscinodiscus*. The results of this study provided the possibility of on-site and *in situ* automatic classification for large batches of *Coscinodiscus* sample images, especially the rapid and effective determination of dominant algae species, which would undoubtedly have good application prospects.

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