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Examination of Phytoplankton Cells by Isolated Cultured Cell Images as Teacher Data

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Abstract: Analysis of the species composition of phytoplankton communities (analysis of community structure), which is indispensable for analysis of photosynthesis and CO_2 absorption, requires long-term image authentication work by skilled researchers. If this work becomes possible through image processing with high accuracy and in large quantities, research on photosynthesis and CO_2 absorption in aquatic ecosystems will markedly advance. In this study, high-quality teacher data are created using isolated cultured cells by biological methods. Using these data, we developed an automatic system to analyze the phytoplankton community structure by deep learning.
Keywords: phytoplankton cells, segmentation, classification, mathematical morphology, convolutional neural networks, deep learning

Introduction

In considering the ecosystem of the Sagami River estuary, it is important to clarify the trends of phytoplankton, which is responsible for primary production[1)]. Determining the species composition of phytoplankton communities and the accurate count of phytoplankton cells in a unit amount of water in different site is critical in evaluating the ecosystem of the Sagami River. This task is usually done manually with time-consuming and labor-intensive efforts of skilled person. This may lead to eye fatigue easily through continuous distinguishing and counting of the cells in microscopic image. With the development of computer vision technology, using automatic counting instead of manual counting becomes urgent. Lots of researchers are resorting on the capabilities of image processing to provide automatic leukocyte segmentation and counting methods based on microscopic blood images[2-8)]. Traditional methods such as thresholding, FCM (Fuzzy C Means), snake and active contour, watershed methods, support vector machine, mathematical morphology and region growing are proposed to improve the identification and counting of cells in the microscope images[9-11)]. But, most of the existing automatic cell counting methods can get the number of cells roughly and usually they are used for calculating the number of certain cell and are difficult to be widely applicable for varied cell image. There is no method for different shapes, size and morphology, and when many different types of cells are mixed in the same image, it is difficult to perform automatic counting of these cells. Nowadays, Machine learning techniques are developed to autonomously learn from mass data and then to identify particular patterns from image with high precision. It is widely used in computer vision tasks such as face detection, face recognition, person tracking, etc. The performance of machine learning heavily rely on annotations of a big set of prepared training data, which is labor-intensive. In this study, we use isolated cultured cell images as teaching data to simplify the annotation process. Then we use one of the "state of the arts" deep learning scheme YOLO (you only look once) to train and classify the phytoplankton cells[12, 13)]. Though

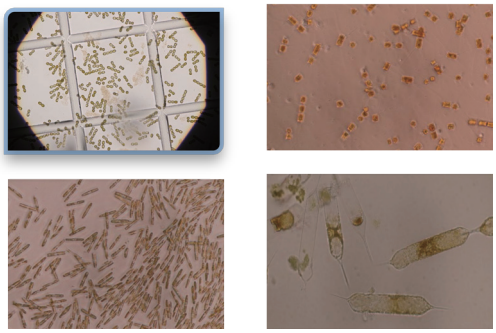

Fig. 1. Samples of homogeneous cells.

the final object is to classify multiple cells from one microscopic image, in the training stage, we can ensure the cells in the image belong to a typical same species by using biological methods. Then we can segment and mark the special cells in the training image through automatic image processing methods. After that, we can train the YOLO network separately for varies phytoplankton cells. Finally, we can combine the pre-trained system into one.

Methods

Acquisition of sample data

There are about 20 dominant species of phytoplankton cells in the Sagami River estuary, each of the cells are with different structures and shapes. It is very difficult to segment and identify the cells when they are mixed together. The collected phytoplankton cells are isolated and cultured for each species from the plankton community, and are incubated for several months to grow one cell into thousands of cells. Then the microscopic images of the homogeneous cells are taken from various angles with fluorescence emitted by UV light, some of the images are taken under natural light. Figure 1 shows a set of samples of the images.

Pre-processing and segmentation

The original images are first divided from 5184 × 3456 into a set of 642 × 432 for the sake of reduction of data size. Then the images are segmented after contrast enhancement, thresholding, noise extraction, augmentation, and morphological modification. The mask images are made for individual images. With the use of these mask images, we can annotate the particular cell images automatically to get the labeled training sets. The steps of the preprocessing are summarized as follows in Fig.2.

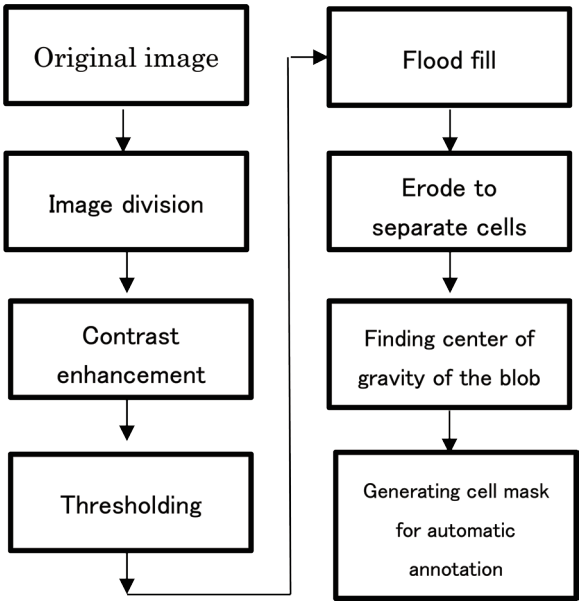

Fig. 2. Pre-processing steps for annotation.

Deep learning by SOLOv3.

Through pre-processing, we can get many cell candidates, but not all of the cells can be extracted from the original image. We will use the parts of the annotated cells to train a network to extract the features for cells of different species. VGGNet, ResNet, U-net, Fast RCNN, Faster RCNN and YOLO are well known deep learning architectures. We have tried some of the above methods and find that YOLOv3 is suitable for our purpose.

YOLOv3 algorithm is an improving algorithm which refers to the network structure of SSD and ResNet, and designs the feature extraction network Darknet-53 with 53 convolution layers as backbone. Originally, the number and value of anchor priors in YOLOv3 are clustered by VOC20 and COCO80 data sets. In this study, we modified the YOLO structure by adaptively training tiny-darknet with our own datasets. First, we train the network with a datasets which contains only one type of cells. Then we add another types of cells to adapt the system which is focused on phytoplankton cells with various shapes or cel under different growing stages. Fig. 3 shows the original network architecture[11]. Then the phytoplankton cells can be detected by the trained YOLOv3 network. Because only one type of cells is existed in the image, the detected cells can be used as training data for further improvement of the YOLOv3 network. The network is then used for detection of unknown images. After detection, it is easy

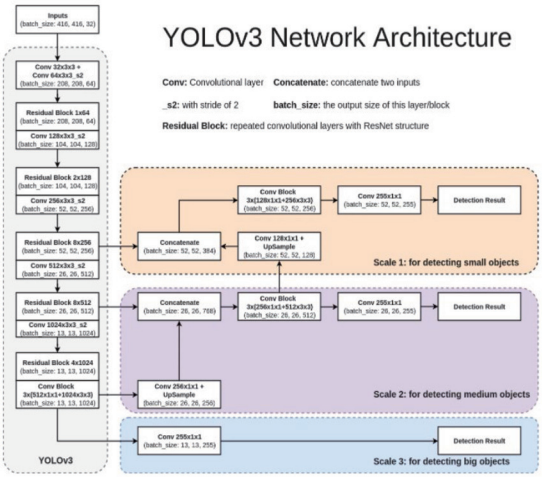

Fig. 3. YOLOv3 Network Architecture.

for us to count the number of the cells in any image with the trained cells.

Results and Discussion

To assess the performance of the methodology, 64 images of the same type of cells are used as training data. Fig. 4A shows an example of the cell image. The image is first transformed into gray scale image, and then it is converted into binary image by Otsu method. The result is shown in Fig. 4B. To fill in the holes in the binary image, we first extend the side of image with dark value, and then perform a flood fill process as shown in Fig. 4C and D. After that, Mathematical morphology filter is applied to erode the cells in the image. As shown in Fig. 4E, the connected cells are separated. The connected white pixels can be viewed as the core of the cell. By computing the moments of each of the connected parts of the image, we can get the gravity center of the candidate cells. Then, we can generate the bounding box of the cells automatically.

Finally, the original image data and the annotation information are used to train the YOLO network. Fig.5 shows some recognition results by the trained YOLOv3 network. We can see that the features of the cell was well caught by the network system. And the YOLO system can be improved by adding the identified cells into the training samples. As for different type of cells, we can also use the same scheme to train the single isolated types of the cells without manual work. In this way, we can collect large number of accurate training data for various types of cells with low labor work.

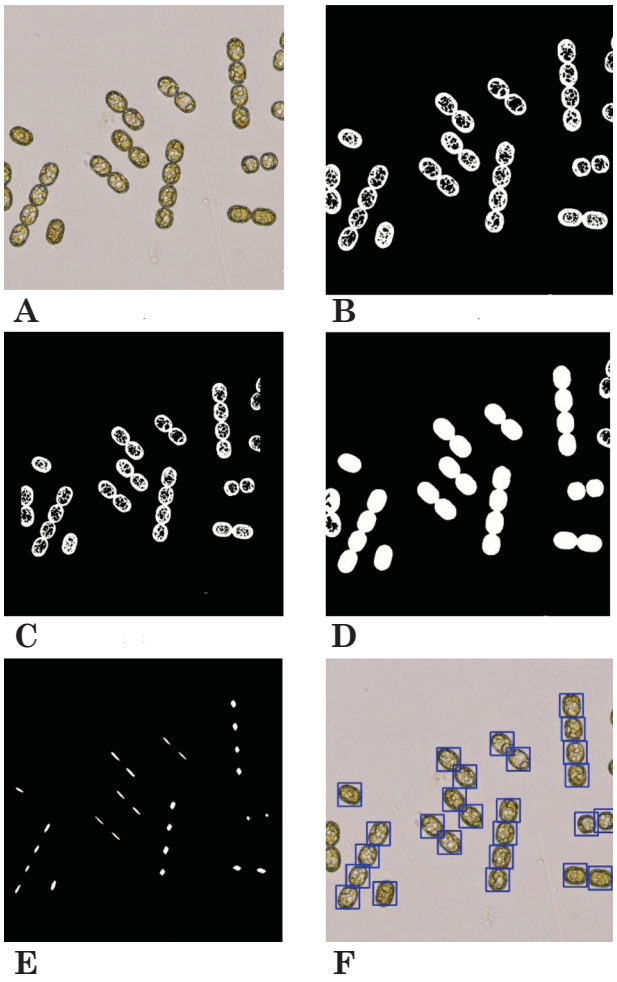

Fig. 4. Automatic annotation.

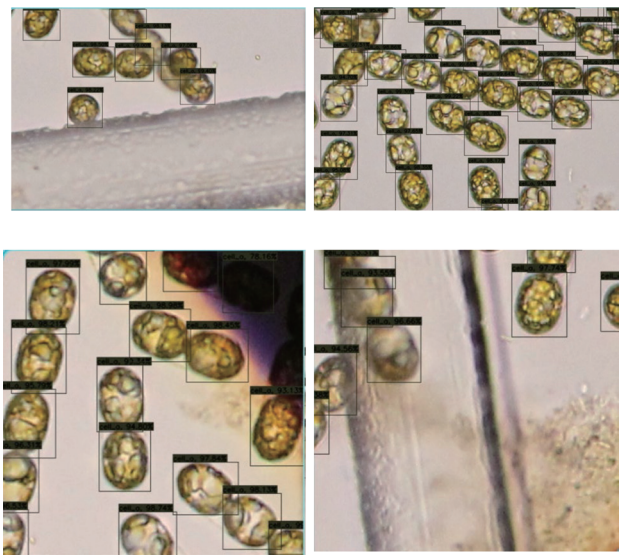

Fig. 5. Cell recognition and counting.

Conclusion remarks

In this paper, we proposed a hybrid methods, which use the biological method and computer vision techniques, to explore the phytoplankton cells in Sagami

River estuary. We take the advance of techniques of the both fields to try to alleviate the burdens of the process. When many different types of cells are mixed together, it is difficult to annotate them automatically. Segmentations of the cells with different textures and shapes may vary for different cell types. Still, the experimental results show the efficiency of the proposed method. In the future, we will complement more segmentation method to automatically generate the bounding box for particular types of cells. Our proposed scheme has a good property for evaluation. When more and more cells are used in the system, the precision of the identification ability will get higher.

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