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Video-Based Real Time Analysis of Plankton Particle Size Spectrum

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ABSTRACT Plankton is one of the most basic components in the marine ecosystem. The community structure and population change of plankton are the important ecological information to reflect the environmental situation. As the fundamental parameter of the plankton community structure, size spectrum is very useful for the evaluation of the marine ecosystem. In this paper, we propose a real-time and adaptive algorithm to calculate the size spectrum of underwater plankton video, which is captured by the high-resolution and high-speed optical camera. First, this algorithm screens the high-resolution plankton images to ensure that every plankton is counted once with the clearest frame. Second, edge detection and morphological methods are performed to get plankton areas. Furthermore, we perform several simplifications that each particle is handled as ellipses shape to calculate the volume to obtain the size spectrum. Moreover, in order to facilitate the biologists to research plankton deeply, we record a region of the clear area containing each plankton to build a plankton database.

INDEX TERMS Underwater vision, clarity index, screening, segmentation, ROI, particle size spectrum.

I. INTRODUCTION

Plankton are the floating communities of plants and animals living in large waters [1]. Plankton lack the ability to move effectively in water, so they typically free-float with water currents. They ubiquitously present across the upper water column in the global ocean [2], [3]. Processes controlling the growth and accumulation of plankton are central to nutrient, carbon, and energy cycling; these provide the foundations for marine and human food webs [4], [5]. Thus, the research on plankton is vital for the stability and biodiversity of marine ecological system [6]. It may not only help people to estimate the climate change and the quality of water, but also contribute to the study of the detailed information of ecological environment and life [7], [8]. However, plankton observation is a complicated and time consuming work. How to facilitate the study on plankton and monitoring their living status has attracted attention from researchers all around the world.

Previously, in order to obtain information of marine plankton, researchers capture samples by on-site sampling using kinds of nets [2], such as Multiple Opening/Closing Net Environmental Sensing System (MOCNESS) [9], Bedford Institute of Oceanography Net Environmental Sensing System (BIONESS) [10], MuiltiNet [11] and Gulf-V [12], etc. Although these methods can obtain the morphological features of plankton, the shortcomings of them are obvious. They all rely on experienced experts to observe and analyze under the microscope on board or at the lab. Microscopic observation is a very time-consuming process. Thus, a method which can monitor the plankton automatically need to be proposed urgently.

Nowadays, optical technology has been applied to study plankton, which provides a useful method for obtaining more detailed information of plankton quickly and accurately. Optical Plankton Counter (OPC) is originally designed by Bedford Institute of Oceanography as a remotely towed sensor providing continuous real time information in size and quantities of plankton [13], [14]. However, it also has some limitations. This method may produce ''coincidence'' phenomenon that two or more particles present in the beam simultaneously and they are counted as one big particle [15]. To solve this problem, Herman developed a new generation of the OPC, that is, the Laser OPC [16].

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However, they can't provide the visual information. To obtain more information on the living status of plankton, people combine plankton research with image analysis techniques, such as Video Plankton Recorder (VPR) [17] and Underwater Video Profiler (UVP) [18], shadowed Image Particle Profiling Evaluation Recorder (SIPPER) [19], Zooplankton Visualization System (ZOOVIS) [20], in-situ Ichthyoplankton Imaging System (ISIIS) [21], Scripps Plankton Camera (SPC) [22], electronic Holographic Camera (eHoloCam) [23], Flow Cytometry, Microscopy (FlowCAM) [24], [25], [27] and Imaging FlowCytobot (IFCB) [26]. FlowCAM [28] is a classical technology of plankton research, which is developed in recent years and has great advantage. By combining with flow cytometry and microscope, this system can obtain the image information of plankton and count them automatically. However, looking through the development of the plankton monitoring, we can find that although the imaging technique gets a lot of developments, the corresponding software which can handle the redundant data and realize really artificial monitoring is lack.

In this paper, we propose a video-based plankton counting algorithm, which can process high-resolution and high-speed plankton video in real time. The rest of this paper is organized as follows. Section II presents the experimental system, including the data sample and optical system. We describe the plankton analysis algorithm in section III. The experimental results are given in section IV. In section V, we present the conclusion and discussion.

II. EXPERIMENT SYSTEM

A. DATA SAMPLING

Our experimental water samples are collected in Plymouth, the United Kingdom. In this paper, we use the water collected by observation stations L4, whose location is $50^o15.00'N$, $4^o13.02'W$, shown as Fig. 1. It is part of the research project ''The Western Channel Observatory (WCO)'' .

B. PLANKTON VIDEO ACQUISITION SYSTEM

Our video acquisition system is mainly composed by a portable Color USB 3.0 camera manufactured by EO company. The size of this camera, $29mm \times 29mm \times 29mm$, makes it to be possible to use in in-situ plankton monitoring, which is our further object. The resolution of the camera is 2048×2048. The corresponding imaging lens is a Mitutoyo Plan Apo Infinity Corrected Long WD Objective with the amplification of $10\times$. Its resolving power is $1.0\mu m$, and the working distance is 33.5mm.

The high resolution and amplification of the whole optical system can meet the demand of clearly imaging microplankton($> 20\mu m$). In order to make the system fit to work in the in-situ situation, different from FlowCam, the imaging system in this paper don't use the flow cytometry framework. The most significant problem of the optical system in this paper is the lack of Flow cell makes it impossible to maintain the object plankton sample be located at the focal plane. In order to achieve effective sampling of living plankton without depth of view control, high speed image acquisition is involved in our system.

FIGURE 1. The location of the water sample. https://www.westernchannelobservatory.org.uk/l4_e1_map.php

By the above video acquisition system, we can get the video information of the sample. However, with high-speed image acquisition, comes the problem of huge amount of data processing and storage. The shooting speed is 80 frames per second in this study. Each image is supposed to be about $800kb$. Thus, the camera may record almost $4.7Gb$ data, which makes it hard to be analyzed in real-time. Moreover, according to the defocus, many duplicate and useless information are also recorded. Several original images are shown in Fig. 2. It is clear in Fig. 2 (a) $\sim$ (c) that except the red box area, the other parts of the images contain defocused unwanted objects. Moreover, the whole image of Fig. 2 (d) is useless, which can be deleted to reduce the use of storage. In order to solve the above problems, specific algorithms is particularly needed.

III. PLANKTON ANALYSIS ALGORITHM

The framework of our algorithm is shown in Fig. 3. In this paper, our work is mainly divided into three steps. In the first step, the plankton images with high-clarity are selected via screening the video with clarity index (discussed in detail in the next subsection). In the second step, the edge detection and image segmentation are applied to the selected original plankton images. We crop the original image with the coordinate values of the outer rectangle of each plankton's connected domain to obtain the required image part with clear plankton. At last, the size spectrum of plankton is calculated

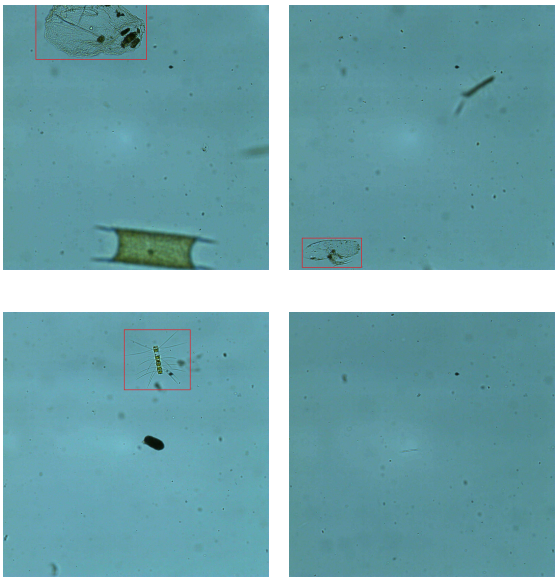

FIGURE 2. Several selected original images.

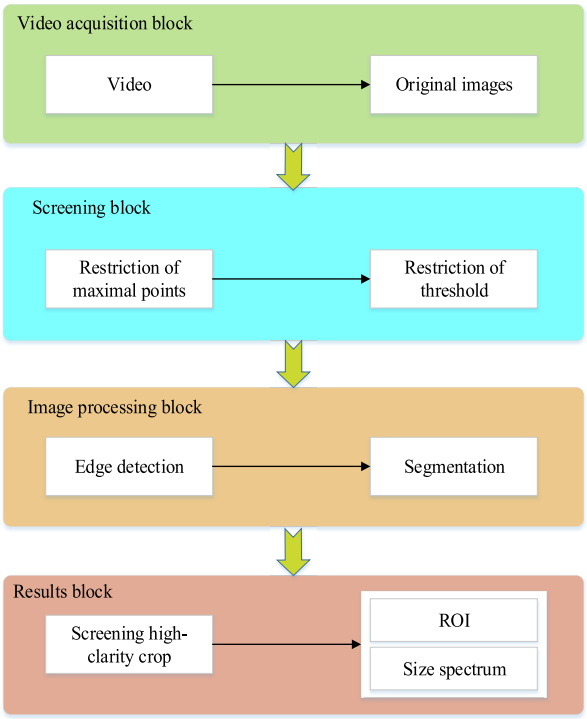

FIGURE 3. The framework of our algorithm.

based on the morphological information of plankton segmented from the second step.

A. SCREENING

In this paper, we use clarity index (CI) to indicate the clarity of one image. Image clarity refers to the sharpness of each detail of the image and its boundary, which is an important index to measure the quality of image. It is coincide with people's subjective feelings better. The low clarity indicates the image is blue, vice versa. In signal processing, the most direct way to distinguish image sharpness is to calculate the fast Fourier transform of the image and observe the high and low frequency components in the image. If there is a small number of high frequency components in the image, this image will be considered as blurred. In this paper, we firstly transform the two-dimensional image function by Laplace transform, which is defined as Eq.1

$$\nabla^2 f(x,y) = f(x+1,y) + f(x-1,y) + f(x,y+1) + f(x,y-1) - 4 \times f(x,y) \quad (1)$$

where, $f(x,y)$ is the original image function, and x, y indicate the coordinates. Then, the variance of the gradient map to get the CI of each image is calculated by the Eq. 2.

$$D(f) = \sum_y \sum_x \left| l(x,y) - \overline{l(x,y)} \right|^2 \quad (2)$$

Here, $l(x,y)$, with average value written as $\overline{l(x,y)}$, is the transformed image function of image $f(x,y)$ by Laplace transform.

Due to the optical system structure, the same target will appear in the obtained video in many continuous frames. We hope that each plankton is counted only once. That is, it is only counted in the clearest frame. However, it is hard to set a threshold defining which is the most clear frame in prior. There are cases in some sequential frames, all the CI is high, as shown in Fig. 4. As we can see in those figures, all of them are very clear. In addition, even if we find a suitable threshold during one period, it is not the optimal threshold for another time period. Thus, we should use an adaptive method to select the needed frame. In this paper, the adaptive screening is performed with the following two restrictions.

(i) We perform Laplace transform on each original image and obtain the CI of each transformed image. According to the change of CI value of each image, we can draw a broken line diagram, which is shown in Fig. 5. As we can see in this figure, the broken line diagram presents the fluctuation change with time. That is to say, the shooting process of living plankton target is along with the clarity changing from low to high and then to low. What we want is the clearest frame during the short period of time. Therefore, we select all the maximal values of the broken line diagram at first.

(ii) Among the first selected maximal points in the first step, there are still many images with low clarity. This is because the maximal points will be generated when no object exists in the imaging field. Thus, we also set an CI threshold to further filter out the frames which do not contain clear plankton. The maximal points below the threshold can be seen as invalid extreme points. In this paper the threshold is set as 3.9 empirically. The threshold line is marked as red in Fig. 5.

B. SEGMENTATION

After the adaptive screening, we get the clear frames containing plankton in the video. Then, we need to detect the edges of the selected frames and get their gradient maps [29], [30]. Because the images are all with high quality now, edge detection is much easier. The gradient maps of original images are first changed into binary images and then be processed

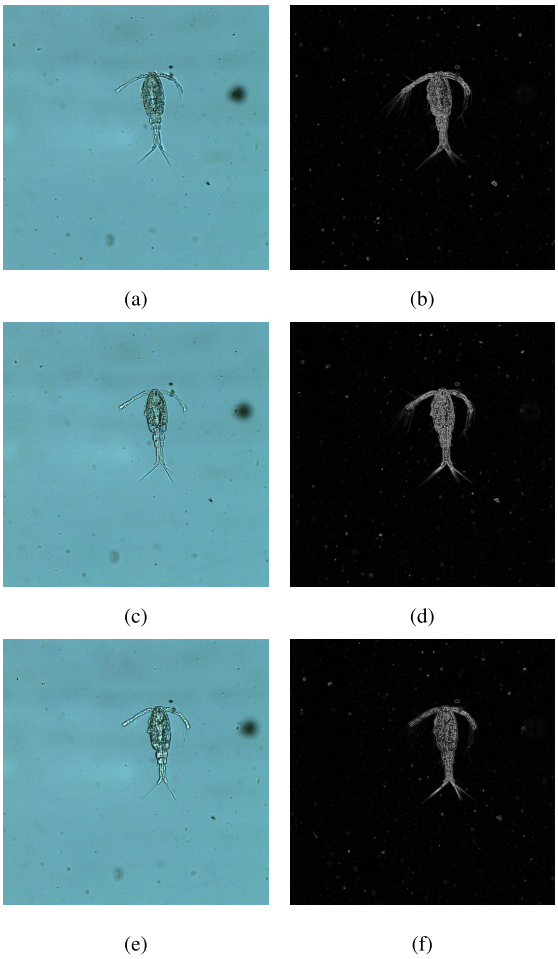

FIGURE 4. (a), (c), (e) are the 152^{th}, 153^{th}, 154^{th} original images, respectively. (b), (d), (f) are the corresponding edge gradient images. (a) CI = 23.0769. (c) CI = 23.0667. (e) CI = 11.6971.

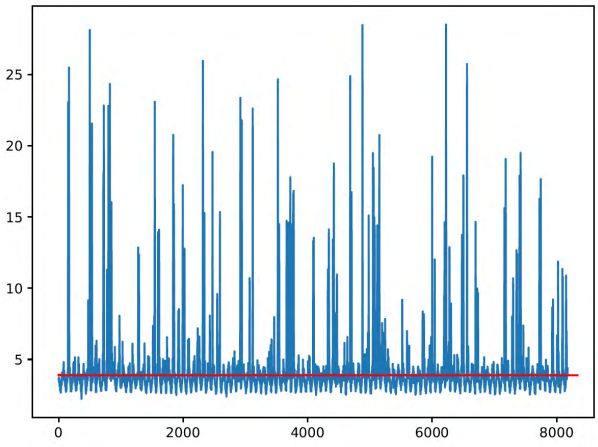

FIGURE 5. The CI change of all frames in this video.

by a series of morphological processes, such as expansion, corrosion and hold filling.

C. ROI

After the process of segmentation, we mark the connected areas in the binary images and get the coordinate values of outer rectangle of each connected area. According to these coordinate values, we can cut out the ROIs of plankton from the selected original images corresponding with binary images. However, there will be low-clarity plankton areas in the screening high-clarity images, as shown in Fig. 6, they also can be segmented. So we need to set a suitable CI threshold to screen all these areas. The area whose CI is higher than this threshold is the clear target area that meet the requirement. When the threshold number is set as 50, our experience can get a better result.

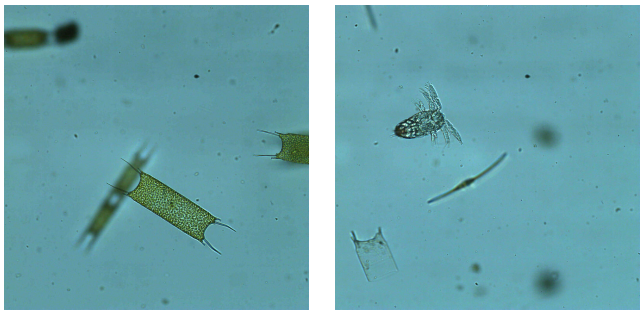

FIGURE 6. Some selected images.

D. PLOT SIZE SPECTRUM

Particle size structure of marine plankton is an important aspect of marine ecological community structure. It can predict the metabolic rate, birth rate and mortality of a community effectively, reflecting the structure and function of marine ecosystem, providing dynamic information on the relationships among the components in the ecosystem. Once plankton in the video are accurately segmented by proposed method, we can use the information of each connected area in binary segmented images to count and compute the final size spectrum in this video [31]. In this paper, in order to obtain the size spectrum, we perform several simplifications that each particle is handled as ellipses-shape to calculate the volume to obtain the size spectra from the two-dimensional image [1]. The volume of each particle (V) is calculated by the Eq. 3. Then we can plot the size spectrum of the plankton in our video.

$$V = \frac{4}{3}\pi abc \tag{3}$$

where, a is the length of the major axis of ellipse that has the same normalized second central moments as the plankton region, b is the length of the minor axis of ellipse that has the same normalized second central moments as the plankton region, and c is equal to b.

IV. EXPERIMENTAL RESULTS

The ROIs of plankton can be accurately cropped, some of those are shown in Fig. 7. These ROIs are of great significance for the establishment of plankton databases and the study of community structure and biodiversity in nature. At the same time, we get the clearest area of each plankton, which can effectively improve the efficiency and accuracy of plankton research. The plankton size spectrum results are shown in Fig. 8(blue). In this figure, the x-axis presents

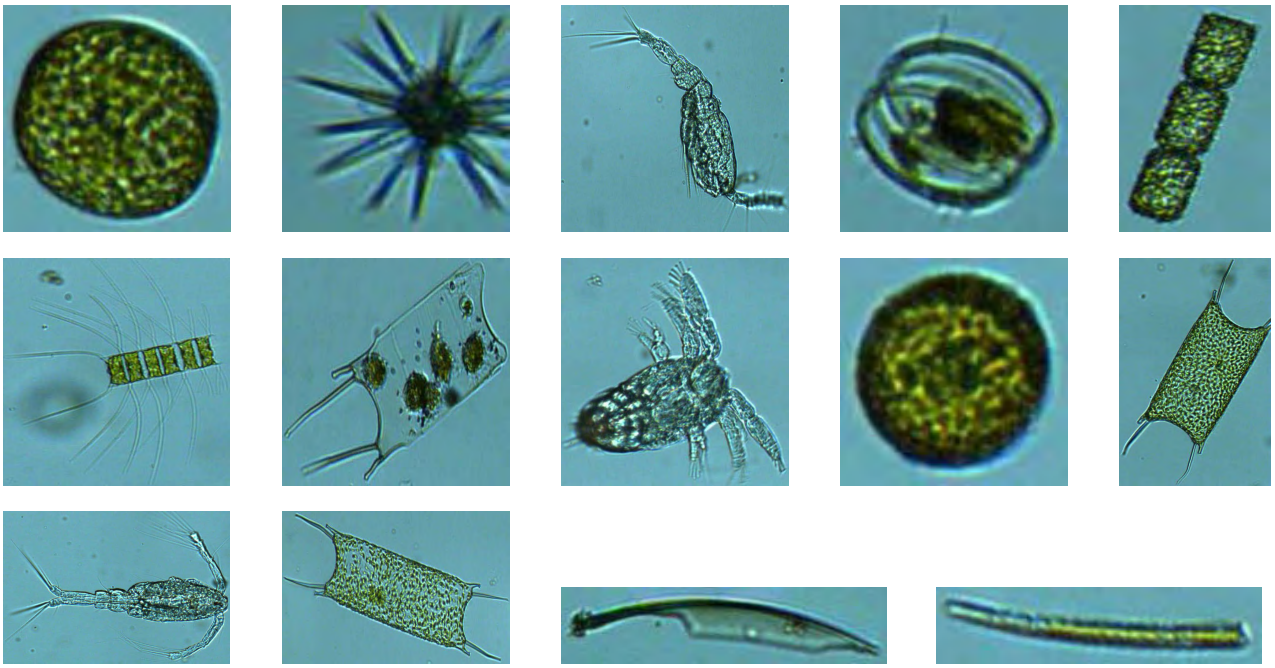

FIGURE 7. A series of ROIs of plankton.

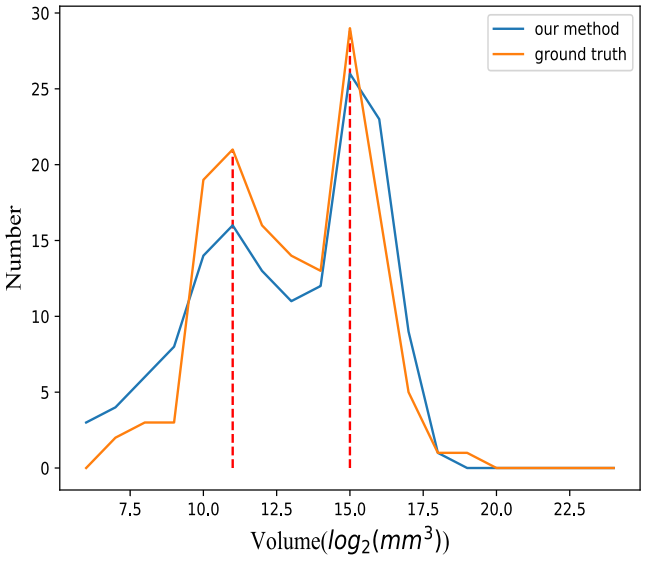

FIGURE 8. The size spectrum of this video calculated by our method (orange) and ground truth (blue).

the volume of the plankton, the unit on x-axis is shown in the logarithm of the volume and 2 is applied as a base. The y-axis presents the number of the plankton. In order to verify the effectiveness of this method, a comparative experiment was carried out. We artificially segment all the clear plankton in this video and every plankton is marked once, then we also plot the size spectrum of labeled images, the ground truth can be shown in Fig. 8(orange). As we can see in this picture, the size spectrum show the double-peak in approximately $11(\log_2(mm)^3)$ and $15(\log_2(mm)^3)$. That's to say, the plankton, with the size of $11(\log_2(mm)^3)$ and $15(\log_2(mm)^3)$ approximately , are predominant in the water sample.

V. DISCUSSION

In this paper, a real-time and adaptive algorithm for calculating plankton size spectrum of the underwater plankton video is proposed. In the algorithm, we use an adaptive method to select the needed frames. Edge detection and morphological methods are used to get plankton areas. Then, several simplifications are used to calculate the volume to obtain the size spectrum. The remarkable advantage of this algorithm is its real-time performance. However, the size spectrum spectra calculated by our algorithm is slightly smaller than the ground truth. This is because that some plankton are omitted from the screening process. In the future, we will optimize our algorithm to achieve higher accuracy and make it used in in-situ plankton monitoring.

REFERENCES

[1] R. W. Sheldon, A. Prakash, and W. H. Sutcliffe, "The size distribution of particles in the ocean," *Limnol. Oceanogr.*, vol. 17, no. 3, pp. 327–340, 1972.

[2] N. Wang, J. Yu, B. Yang, H. Y. Zheng, and B. Zheng, "Vision-based *in situ* monitoring of plankton size spectra via a convolutional neural network," *IEEE J. Ocean. Eng.*, to be published.

[3] S. D. Batten, E. S. Fileman, and E. Halvorsen, "The contribution of microzooplankton to the diet of mesozooplankton in an upwelling filament off the north west coast of Spain," *Prog. Oceanogr.*, vol. 51, nos. 2–4, pp. 385–398, Nov. 2001.

[4] C. A. Vargas and H. E. González, "Plankton community structure and carbon cycling in a coastal upwelling system. I. Bacteria, microprotozoans and phytoplankton in the diet of copepods and appendicularians," *Aquatic Microbial Ecol.*, vol. 34, no. 2, pp. 151–164, 2004.

[5] C. B. Miller and P. A. Wheeler, *Biological Oceanography*. Hoboken, NJ, USA, Wiley, 2012.

[6] H. Lu, D. Wang, Y. Li, J. Li, and I. Humar, "CONet: A cognitive ocean network," 2019.

[7] H. Lu, Y. Li, M. Chen, H. Kim, and S. Serikawa, "Brain intelligence: Go beyond artificial intelligence," *Mobile Netw. Appl.*, vol. 23, no. 2, pp. 368–375, 2018.

[8] H. Lu, Y. Li, T. Uemura, H. Kim, and S. Serikawa, "Low illumination underwater light field images reconstruction using deep convolutional neural networks," *Future Gener. Comput. Syst.*, vol. 82, pp. 142–148, May 2018.
[9] P. H. Wiebe *et al.*, "New development in the MOCNESS, an apparatus for sampling zooplankton and micronekton," *Mar. Biol.*, vol. 87, no. 3, pp. 313–323, 1985.
[10] D. D. Sameoto, L. O. Jaroszynski, and W. B. Fraser, "BIONESS, a new design in multiple net zooplankton samplers," *Can. J. Fisheries Aquatic Sci.*, vol. 37, no. 4, pp. 722–724, 1980.
[11] H. Weikert and H.-C. John, "Experiences with a modified Bé multiple opening-closing plankton net," *J. Plankton Res.*, vol. 3, no. 2, pp. 167–176, 1981.
[12] D. Sameoto *et al.*, "Collecting zooplankton," *ICES Zooplankton Methodol. Manual*, vol. 40, no. 2, pp. 55–81, 2000.
[13] A. W. Herman, "Simultaneous measurement of zooplankton and light attenuance with a new optical plankton counter," *Continental Shelf Res.*, vol. 8, no. 2, pp. 205–221, 1988.
[14] A. W. Herman, "Design and calibration of a new optical plankton counter capable of sizing small zooplankton," *Deep Sea Res. A, Oceanogr. Res. Papers*, vol. 39, nos. 3–4, pp. 395–415, 1992.
[15] A. Remsen, T. L. Hopkins, and S. Samson, "What you see is not what you catch: A comparison of concurrently collected net, optical plankton counter, and shadowed image particle profiling evaluation recorder data from the northeast gulf of mexico," *Deep Sea Res. I, Oceanogr. Res. Papers*, vol. 51, no. 1, pp. 129–151, 2004.
[16] A. W. Herman, B. Beanlands, and E. F. Phillips, "The next generation of optical plankton counter: The laser-OPC," *J. Plankton Res.*, vol. 26, no. 10, pp. 1135–1145, 2004.
[17] C. S. Davis, F. T. Thwaites, S. M. Gallager, and Q. Hu, "A three-axis fast-tow digital video plankton recorder for rapid surveys of plankton taxa and hydrography," *Limnol. Oceanogr. Methods*, vol. 3, no. 2, pp. 59–74, 2005.
[18] M. Picheral, L. Guidi, L. Stemmann, D. M. Karl, G. Iddaoud, and G. Gorsky, "The underwater vision profiler 5: An advanced instrument for high spatial resolution studies of particle size spectra and zooplankton," *Limnol. Oceanogr. Methods*, vol. 8, no. 9, pp. 462–473, 2010.
[19] S. Samson, T. Hopkins, A. Remsen, L. Langebrake, T. Sutton, and J. Patten, "A system for high-resolution zooplankton imaging," *IEEE J. Ocean. Eng.*, vol. 26, no. 4, pp. 671–676, Oct. 2001.
[20] M. C. Benfield, C. J. Schwehm, and S. F. Keenan, "ZOOVIS: A high resolution digital camera system for quantifying zooplankton abundance and environmental data," *Amer. Soc. Limnol. Oceanogr.*, pp. 12–17, 2001.
[21] R. K. Cowen and C. M. Guigand, "*In situ* ichthyoplankton imaging system (ISIIS): System design and preliminary results," *Limnol. Oceanogr. Methods*, vol. 6, no. 2, pp. 126–132, 2008.
[22] J. S. Jaffe, "To sea and to see: That is the answer," *Methods Oceanogr.*, vols 15–16, pp. 3–20, Apr./Jul. 2016.
[23] H. Sun, D. C. Hendry, M. A. Player, and J. Watson, "*In situ* underwater electronic holographic camera for studies of plankton," *IEEE J. Ocean. Eng.*, vol. 32, no. 2, pp. 373–382, Apr. 2007.
[24] C. K. Sieracki, M. E. Sieracki, and C. S. Yentsch, "An imaging-in-flow system for automated analysis of marine microplankton," *Mar. Ecol. Prog.*, vol. 168, no. 1, pp. 285–296, 1998.
[25] K. Ide, K. Takahashi, K. Kuwata, M. Nakamachi, and H. Saito, "A rapid analysis of copepod feeding using FlowCAM," *J. Plankton Res.*, vol. 30, no. 3, pp. 275–281, 2008.
[26] R. J. Olson and H. M. Sosik, "A submersible imaging-in-flow instrument to analyze nano- and microplankton: Imaging flowcytobot," *Limnol. Oceanogr. Methods*, vol. 5, no. 6, pp. 195–203, 2007.
[27] J. H. See, L. Campbell, J. L. Pinckneyand T. L. Richardson, R. Shen, and N. L. Guinasso, "Combining new technologies for determination of phytoplankton community structure in the northern gulf of mexico," *J. Phycol.*, vol. 41, no. 2, pp. 305–310, 2005.
[28] E. J. Buskey and C. J. Hyatt, "Use of the FlowCAM for semi-automated recognition and enumeration of red tide cells (Karenia brevis) in natural plankton samples," *Harmful Algae*, vol. 5, no. 6, pp. 685–692, 2006.
[29] H. Lu, Y. Li, S. Mu, D. Wang, H. Kim, and S. Serikawa, "Motor anomaly detection for unmanned aerial vehicles using reinforcement learning," *IEEE Internet Things J.*, vol. 5, no. 4, pp. 2315–2322, Aug. 2018.
[30] S. Serikawa and H. Lu, "Underwater image dehazing using joint trilateral filter," *Comput. Elect. Eng.*, vol. 40, no. 1, pp. 41–50, Jan. 2014.
[31] R. Wang and Z. Zhang, "A Study on the size spectra of benthos," *Trans. Oceanol. Limnol.*, vol. 4, pp. 61–68, Jan. 2003.

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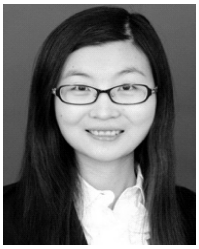

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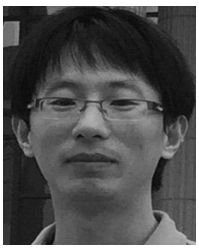

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