Detection of viability of micro-algae cells by optofluidic hologram pattern

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A rapid detection of micro-algae activity is critical for analysis of ship ballast water. A new method for detecting micro-algae activity based on lens-free optofluidic holographic imaging is presented in this paper. A compact lens-free optofluidic holographic imaging device was developed. This device is mainly composed of a light source, a small through-hole, a light propagation module, a microfluidic chip, and an image acquisition and processing module. The excited light from the light source passes through a small hole to reach the surface of the micro-algae cells in the microfluidic chip, and a holographic image is formed by the diffraction light of surface of micro-algae cells. The relation between the characteristics in the hologram pattern and the activity of micro-algae cells was investigated by using this device. The characteristics of the hologram pattern were extracted to represent the activity of micro-algae cells. To demonstrate the accuracy of the presented method and device, four species of micro-algae cells were employed as the test samples and the comparison experiments between the alive and dead cells of four species of micro-algae were conducted. The results show that the developed method and device can determine live/dead microalgae cells accurately. *Published by AIP Publishing.* <https://doi.org/10.1063/1.5021179>

I. INTRODUCTION

Ship transportation is one of the most important ways for international trade. Over 90% of the world's goods are transported by ship owing to its high carrying capacity and low costs. Ships employed for international trade and transportation need to carry ballast water to keep the ship's balance. However, the ballast water has also become a main way of foreign biological invasion.¹ Approximately 10×10^9 tons of ballast water are switched over worldwide every year and about 3000 species of organisms migrate with ballast water every day. The foreign biological invasion caused by ballast water has brought a great threat to the global biodiversity and ocean ecological environment.^{2–4} Micro-algae cells with a size range of $10 \mu\text{m}$ – $50 \mu\text{m}$ are the main organisms in the ballast water.⁵ Many treatment systems for killing the living micro-algae cells in ballast water have been developed in the past few years.^{6–8} The detection of activity of micro-algae cells plays an important role in assessing the performance of the treatment systems. Therefore, *on-site* fast analysis of micro-algae cells is necessary for the port countries.

The most widely used method of detecting the activity of micro-algae cells is still the use of microscope, which requires professional personnel to operate the instrument and often involves errors in the determination of activity of micro-algae cells. Furthermore, the microscope method is time consuming and labor intensive to obtain an accurate result. Many

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automatic methods of detecting the activity of micro-algae cells have been developed including flow cytometry, Polymerase Chain Reaction (PCR), molecular recognition genomics, and so on.^{9–14} These methods can determine the activity of micro-algae cells accurately. However, high cost, complex operation, and large volume severely limit the use of these instruments in the *on-site* detection of micro-algae cells in ship ballast water.

Microfluidic, optofluidic, or Lab-on-chip technologies are very promising means for micro total analysis, and have been widely researched in many fields such as chemistry, biology, medicine, environment, and ships and ocean engineering.^{15–27} Some microfluidic methods of detection of micro-algae cells activity have been studied in the past few years.^{28–34} High throughput and fast detection are still the challenge for analysis of micro-algae cells in a large volume of ballast water.

A lens-free optofluidic holographic imaging technology has been presented for several years and it is a method combining microfluidic technology and optical imaging technology.^{35–39} Compact optical path and abundant holographic features associated with the internal characteristics of microparticles make this method especially useful for *on-site* rapid detection.

This paper reports a new method for detecting the activity of micro-algae cells based on lens-free optofluidic holographic imaging. A microfluidic chip was used as the sample platform and the lens-free optofluidic holographic imaging device was set up by combining the microfluidic chip with holographic imaging technology. The characteristics of the hologram pattern of the micro-algae cells were extracted and analyzed, and employed to indicate the activity of the micro-algae cells. The comparison experiments of four species of living and dead micro-algae cells were performed by using the developed device.

II. METHOD AND MATERIALS

A. Principle of micro-algae cell activity represented by hologram pattern

In the lens-free optofluidic holography device developed in this study, the schematic of hologram pattern formation is shown in Fig. 1(a). The light source irradiates the micro-algae cells in a microfluidic chip through a small hole. The scattered light traveling through the micro-algae cells (object wave) interferes with the light traveling straight through the microfluidic chip (reference wave, which does not pass through any of microalgae cells) and forms the hologram pattern, which is recorded by a CCD sensor. The hologram pattern depends on many different geometrical properties of cells, e.g., its refractive index, its thickness and general shape, and the position of the cell in the microfluidic chip. When there are changes in the activities of microalgae cells, the characteristics of the microalgae cells might change and result in changes in the hologram pattern. However, a comprehensive physical model is hard to be established to describe the relation between the hologram pattern and these complex properties of cells.

To show the relation between the hologram pattern and the properties of the microalgae cells more clearly, refractive index as a typical characteristic to represent the inner properties of microalgae cells is chosen as an example and illustrated to show how the changes in the refractive index affect the changes in the hologram pattern. The schematic of relation module between the refractive index and the hologram pattern is shown in Fig. 1(b). In this simplified physical model, only refractive index is considered and other properties are assumed to be constant.

The refractive index of a micro-algae cell is the ratio of the speed of light in vacuum to that in the micro-algae cell. The higher the refractive index of a micro-algae cell is, the stronger the incident light in the cell is refracted. The refractive index of a micro-algae cell is mainly determined by cellular substances contents, sizes, shapes, and distributions. When the activity of a micro-algae cell changes, the internal substances and external structures of the cell will change. The contents of cellular active substances such as pigment, Chlorophyll A, and enzyme decrease and the structures of cell membrane and cell wall change and even shrink. These changes will result in change in the refractive index of the micro-algae cell. Furthermore, when the activity of a micro-algae cell decreases, the contents of cellular internal pigments and enzymes reduce, and thus the electromagnetic field in the cell attenuates and the interaction between the charged particles and internal electromagnetic field in the cell is weakened, and

(a)

(b)

FIG. 1. (a) Schematic of hologram pattern formation in our developed system; (b) schematic of relation module between the refractive index and the hologram pattern of micro-algae cells.

consequently, the speed of incident light in the cell becomes slower and the refractive index of the micro-algae cell becomes larger.

The refraction indexes of the micro-algae system correlate each other via Snell's Law as follows:

$$n_c \sin \theta_1 = n_s \sin \theta_{cb}, \quad (1)$$

$$n_c \sin \theta_2 = n_s \sin \theta_{fd}, \quad (2)$$

where n_c is the refractive index of micro-algae cells, n_s is the refractive index of cells culture solution, and θ_1 and θ_{cb} are the incident angle and the diffraction angle of the central bright speckle of hologram pattern of micro-algae cells, respectively. θ_2 and θ_{fd} are the incident angle and the diffraction angle of the first dark stripe of hologram pattern of micro-algae cells, respectively. The incident angles θ_1 and θ_2 remain unchanged when the position of the light source is fixed. The refractive index n_s of cells culture solution is constant. When the activity of a micro-algae cell decreases, the refractive index n_c of the micro-algae cell becomes larger, and thus the diffraction angles θ_{cb} and θ_{fd} of hologram pattern of the micro-algae cell become larger.

In Fig. 1, the radii r_{fd} and r_{cb} of the central bright speckle and first dark stripe of hologram pattern of the micro-algae cell can be expressed as follows:

$$r_{cb} = Z_2 \cdot \tan \theta_{cb}, \quad (3)$$

$$r_{fd} = Z_2 \cdot \tan \theta_{fd}, \quad (4)$$

where Z_2 is the distance from the micro-algae cells to the CCD sensor plane. Thus, when the activity of a micro-algae cell decreases, the diffraction angles θ_{cb} and θ_{fd} of hologram pattern of the micro-algae cell get larger, and the radii r_{fd} and r_{cb} of the central bright speckle and first dark stripe of hologram pattern of the micro-algae cell become larger, and the areas of the central bright speckle and first dark stripe of hologram pattern of the micro-algae cell become larger.

In addition, the light intensity difference between the bright and dark stripes in the hologram pattern of micro-algae cells can be expressed by the contrast ratio K as follows:

$$K = (I_M - I_m)/(I_M + I_m), \quad (5)$$

where I_M and I_m are the maximum and minimum of the light intensity in the hologram pattern, respectively. According to the theory of diffraction,⁴⁰ the light intensity of hologram pattern of a micro-algae cell is mainly related to the wavelength and intensity of the excitation light, the contents of the active substances, the size, and structure of the micro-algae cell. When the activity of a micro-algae cell decreases, the active substances in the cell reduce, and the permeability of the cell membrane increases, and the light intensity of the hologram pattern becomes larger. The bright stripes in the hologram pattern become brighter and the dark stripes become darker, and the contrast ratio K becomes larger. The difference in the gray values between the central bright speckle and the first dark stripe in hologram pattern becomes larger.

B. Lens-free optofluidic holographic imaging system

1. System design

The schematic of the developed lens-free optofluidic holographic imaging system is shown in Fig. 2. The system is composed of a LED light source, a light aperture, a microfluidic chip, a lens-free CCD sensor, and an image acquisition and processing module. The excitation light with 650 nm central wavelength emits from a LED light source (LZ1-00R105, LedEngin, USA). To meet requirements for the excitation light in hologram imaging, partially coherent light is obtained by passing LED light through a light aperture. The diameter of the light aperture in this system is about 130 μm . An optical transmission structure was designed to prevent the interference from ambient light and improve the quality of the hologram pattern. The partially coherent light propagating along the optical transmission structure is used to irradiate micro-algae cells in a microfluidic chip. A commercial CCD sensor (MT9P031V3, APTINA, USA) without its lens is used to detect the hologram pattern. The distance between the light aperture and micro-algae cells plane in the microfluidic chip is represented by the symbol Z_1

FIG. 2. Schematic of lens-free optofluidic holographic imaging system.

and the distance between the cells plane and CCD sensor plane is represented by Z_2 . To meet the requirements of holographic imaging, Z_1 is about 5 cm and Z_2 is about 2 mm in our developed system. When the partially coherent light excites the micro-algae cells, the formed hologram patterns can be recorded without distortion by CCD sensor without lens and transmitted to PC through a data acquisition board (USB2.0, XIANMISI, China). Thus, the characteristics of the hologram pattern can be analyzed and the activities of micro-algae cells can be determined.

2. Design and fabrication of the microfluidic chip

The structure diagram of the microfluidic chip used in this study is shown in Fig. 3. This microfluidic chip consists of a sample reservoir, a waste reservoir, a focusing microchannel and an imaging region. The width and the length of the microchannel from the sample reservoir to the imaging region are $500\ \mu\text{m}$ and 5 mm, respectively. A focusing microchannel with a gradually reduced cross-section allows the micro-algae cells to flow into the imaging region one by one (to avoid cells aggregation). The end width and the length of the focusing microchannel $50\ \mu\text{m}$ and $100\ \mu\text{m}$ and the imaging region is $500\ \mu\text{m}$ wide and $500\ \mu\text{m}$ long. The microchannels have a height of $40\ \mu\text{m}$ and the reservoirs have a diameter of 3 mm and a depth of 2 mm. The microfluidic chip was made of a layer of polydimethylsiloxane (PDMS) and a piece of glass slide following the standard soft-lithography process.⁴¹ A SU8 master was made through coating, post-baking and developing processes. A PDMS replica was peeled off from the master after the mixture of PDMS and curing agent were mixed, degassed, poured on the master, and heated in the vacuum oven. The PDMS layer with the microchannel structure and wells was bonded onto a glass slide after they were treated by oxygen plasma in a plasma cleaner.

FIG. 3. Structure diagram of the designed microfluidic chip.

C. Preparation of samples

1. Culture of micro-algae cells

Four species of micro-algae cells (*Platymonas subcordiformis*, *Pyramimonas* sp., *Closterium lunula*, and *Isochrysis* sp.) were taken as samples to be tested. These micro-algae cells are provided by the Liaoning Sea Fisheries Research Institute (LSFRI). Each species of micro-algae cells is cultivated separately in seawater in a flask. The flask is put into a carbon dioxide incubator (MGC-300A, Yiheng Technical Co., Ltd., Dalian Maritime University, Dalian, China) at a temperature of 22 °C and shaken once every three hours during the day.

2. Inactivation of micro-algae cells

A volume of 1 ml of the micro-algae cells solution was placed in a 1.5 ml centrifuge tube. The tube was then placed in a 50 ml beaker. 35 ml of pure water was added into the beaker placed on a heater, and the temperature of the micro-algae cells solution was gradually increased to 100 °C for 15 min. The solution was then cooled to room temperature. A microscope was employed to check if all micro-algae cells were inactive to make sure that all micro-algae cells had died.⁴²

III. RESULTS AND DISCUSSION

A. Analysis of characteristics of hologram pattern of micro-algae cells

As typical examples, two holographic images of the living cells and the dead cells of *Platymonas subcordiformis* are shown in Fig. 4. The enlarged view shows that the holographic image is made up of a series of alternating bright and dark circular stripes. The central bright speckle and the first dark stripe are the obvious features in the holographic image. To show these two features more clearly, the white dotted lines were used to mark them in Fig. 4. It can be seen that some characteristics in the hologram pattern are different between living and dead cells. For example, by comparing the images in Fig. 4, one can see that the size and the gray level of the central bright speckle and the first dark stripe are different between the alive cells and the dead cells. That means that these characteristics are related to the activities of the micro-algae cells. Therefore, the sizes and gray values of the central bright speckle and the first dark stripe in hologram pattern were analyzed quantitatively, the results are listed in Table I. The area of the central bright speckle is expressed by A_{cb} , and A_{fd} means the area of the first dark stripe. The gray value of the central bright speckle G_{cb} and that of the first dark stripe G_{fd} represent the average of the gray values in the central bright speckle and the first dark stripe, respectively. From Table I, A_{cb} and A_{fd} are different for the living and dead cells, and the central bright speckle area A_{cb} of living micro-algae cells is smaller than that of the dead micro-algae cells. The average gray value G_{cb} of

FIG. 4. (a) The holographic images of the living micro-algae cells (*Platymonas subcordiformis*). (b) The holographic images of the dead micro-algae cells (*Platymonas subcordiformis*).

the central bright speckle is smaller than that of dead cells while the average gray value G_{fd} of the first dark stripe is higher than that of the dead cells. In Table I, D_{gray} is the difference between G_{cb} and G_{fd} . All these parameters or the combination of these parameters can be taken as the indicator parameters of the activity of the micro-algae cells.

TABLE I. The typical parameters in holographic pattern of the micro-algae cells (*Platymonas subcordiformis*).

Typical parameters of hologram pattern	<i>Platymonas subcordiformis</i>	
	Living cells	Dead cells
A_{cb} (area of the central bright speckle)	78.85	93.40
G_{cb} (average of gray values of central bright speckle)	110.86	113.01
A_{fd} (area of the first dark stripe)	287.15	342.60
G_{fd} (average of gray values of the first dark stripe)	94.33	92.00
D_{gray} (gray level difference between G_{cb} and G_{fd})	16.53	20.97

B. Comparison experiments of different micro-algae cells

In order to investigate the general applicability of the presented method, another three different species of micro-algae cells (*Pyramimonas* sp., *Closterium lunula*, and *Isotopic* sp.) were taken as the test samples, and the comparison experiments between the living micro-algae cells and the dead micro-algae cells were performed. The results of three species of microalgae cells of *Pyramimonas* sp., *Closterium lunula*, and *Isotopic* sp. are summarized in Figs. 5(a), 5(b), and 5(c), respectively. The results of every parameter in Fig. 5 are all the average value of twenty hologram patterns. The results from Fig. 5(a) show that the hologram patterns of the living cells and the dead cells of *Pyramimonas* sp. are different. The parameters extracted from hologram

FIG. 5. Parameters analysis of hologram pattern of the micro-algae cells: (a) *Pyramimonas* sp., (b) *Closterium lunula*, and (c) *Isotopic* sp.

FIG. 6. Judgment of liveness and death of the micro-algae cells by D_{gray} in hologram pattern.

patterns of *Platymonas subcordiformis* cells (Fig. 4 and Table I) are applicable for *Pyramimonas sp.* microalgae cells. The hologram patterns of the other two species microalgae cells *Closterium lunula* and *Isotypic sp.* in Figs. 5(b) and 5(c) have also the same features. In general, the central bright speckle area A_{cb} of the living micro-algae cells is smaller than that of the dead micro-algae cells. The average gray value of the central bright speckle G_{cb} of the living cells is also smaller than that of the dead cells. The average gray value G_{fd} of the first dark stripe of the living cells is also higher than that of the dead cells. Particularly, as seen from Figs. 5(a), 5(b), and 5(c), the parameter A_{fd} is very sensitive and shows a significant difference between the living micro-algae cells and the dead micro-algae cells. This is mainly because the change in the activities of micro-algae cells causes the change in light intensity distribution in the hologram pattern and results in the change in areas and gray values between the central bright speckle and the first dark stripe in the hologram pattern.

In addition, it was found that the parameter D_{gray} is more sensitive to distinguish the activity of micro-algae cells than A_{cb} and G_{cb} . As shown in Fig. 6, a certain threshold value of D_{gray} can be used to judge the liveness or death of the same species of micro-algae cells. In summary, the parameters A_{fd} and D_{gray} can be employed to determine the liveness or death of micro-algae cells for *on-site* analysis of ballast water.

IV. CONCLUSION

This is the first time that a lens-free optofluidic holographic imaging method is applied to obtain characteristics of the activity of micro-algae cells. The relationship between characteristics of hologram pattern and activity of micro-algae cells was analyzed. Comparison experiments of four species of the alive micro-algae cells and the dead micro-algae cells were conducted. The results show that the extracted parameters from hologram patterns can be employed to evaluate the activity of micro-algae cells, especially for judging the liveness and death of micro-algae cells. Particularly, the area of the first dark stripe A_{fd} of the dead cells is larger than that of the living cells. Furthermore, the gray level difference between the central bright speckle and the first dark stripe is larger for the dead cells than that of the living cells. The presented method has the advantages of fast detection, compact size, and low cost and is particularly useful for *on-site* analysis of micro-algae cells in ship ballast water.

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