Digital holographic camera for plankton monitoring

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ABSTRACT

A submersible digital holographic camera for measuring plankton and other particles is described. The camera provides underwater recording of digital holograms of water volume containing plankton followed by automatic restoration of holographic images of plankton species, determination of their sizes, shapes, and concentrations, and their recognition and classification. Particles with sizes of 200 µm and larger are analyzed. The water volume registered per exposure is about 1 L. The special features of the software for automatic information retrieval from digital holograms are discussed. Examples of application of the camera as an integral part of the hardware-software complex for field measurements are given. Prospects for application of this complex for ecological monitoring are discussed. The recognition criterion of the digital holographic camera and the data volume and the averaging time required for obtaining statistically reliable data on plankton species are also given.

Keywords: plankton, digital holography, resolution

1. INTRODUCTION

The plankton in the world ocean is an important factor of biodiversity conservation, and its use as a bioindicator in the habitat allows the stability of the environment against the action of various unfavorable factors associated, for example, with human economic activity to be estimated. By virtue of this, a study of the plankton diversity is one of the main components of monitoring of the world ocean. In the process of monitoring of water areas in regions of human economic activity (mining sites, oil platforms, ports, and gas pipelines), a problem arises of studying other particles as well, including settling particles of various origin, oil drops, and gas bubbles.

To solve these problems, we suggest the DHC technology based on the application of the digital holographic camera [1–5] that allows data on the plankton and other particles to be obtained without sampling. In this case, we obviate the necessity of the routine and labor-consuming stage of plankton trapping, fixation, and storage and processing of samples of plankton or other particles *in vitro*.

The DHC technology suggested here includes *in situ* recording of digital holograms of the medium with subsequent automatic restoration of holographic images of plankton species and other particles, determination of their sizes, shapes, concentrations, their recognition, and classification. A distinctive feature of our technical solution is the volume of the medium investigated per exposure equal approximately to 1 L.

In the present work, technical features and capabilities of the developed complex are considered based on materials of field sea missions in different water areas. In this case, the main problem in recognition and further classification of particles is the recognition of particle shapes; to this end, laboratory and field data are analyzed in the present study.

2. INTRODUCTION

Figure 1 shows optical schemes of two DHC modifications – with linear radiation passage through the investigated volume of the medium (a) and with folded optical system (b) [1, 2]. For brevity, below we call the first realization linear, and the second realization folded. The last scheme is used to reduce the overall dimensions of the complex and to provide quite large investigated volume. The folded scheme is provided by a mirror and prism system (prism 6 in Fig. 1).

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At the same time, both schemes are intended for in-line digital hologram recording. Indeed, light from laser source (1) passes in both schemes through a collimator, forms the beam with desired cross section, passes through the investigated volume with particles and the receiving objective, and is then incident at CCD (CMOS) camera (5). In this case, as it is typical for in-line scheme, the part of radiation scattered by the particles represents the objective wave, and the part of radiation passed by the particles is the reference wave. As a result, camera (5) records the interference pattern of these two coherent waves, which is the digital hologram of the investigated volume.

This digital hologram represents a two-dimensional discrete array of quantized intensities for the interference pattern formed by the reference and objective waves and is subsequently used as the initial field distribution for restoration of the image of the investigated volume.

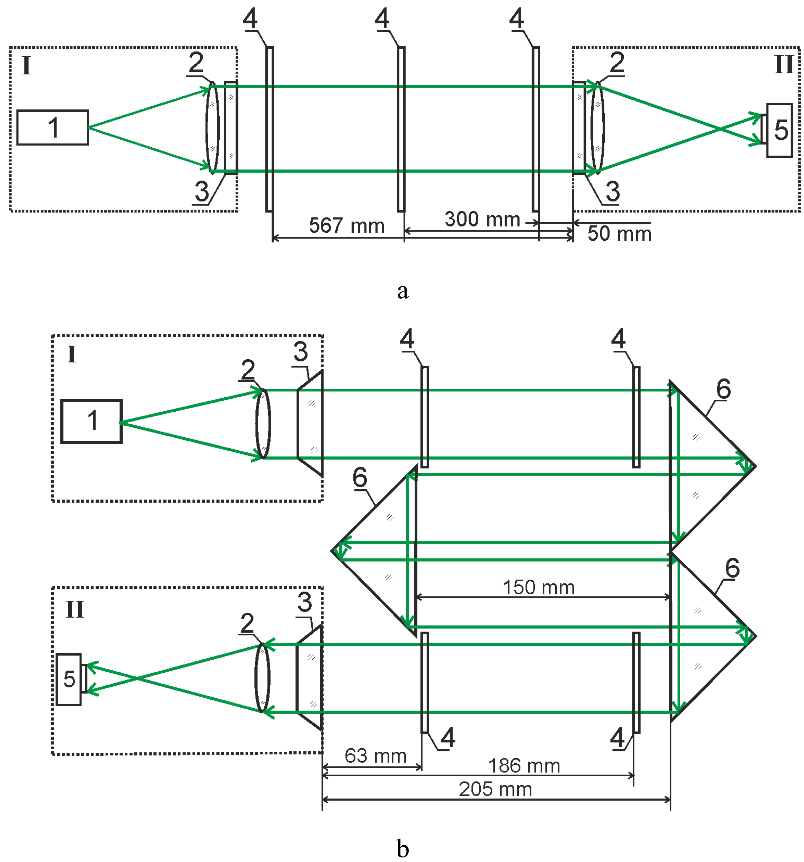
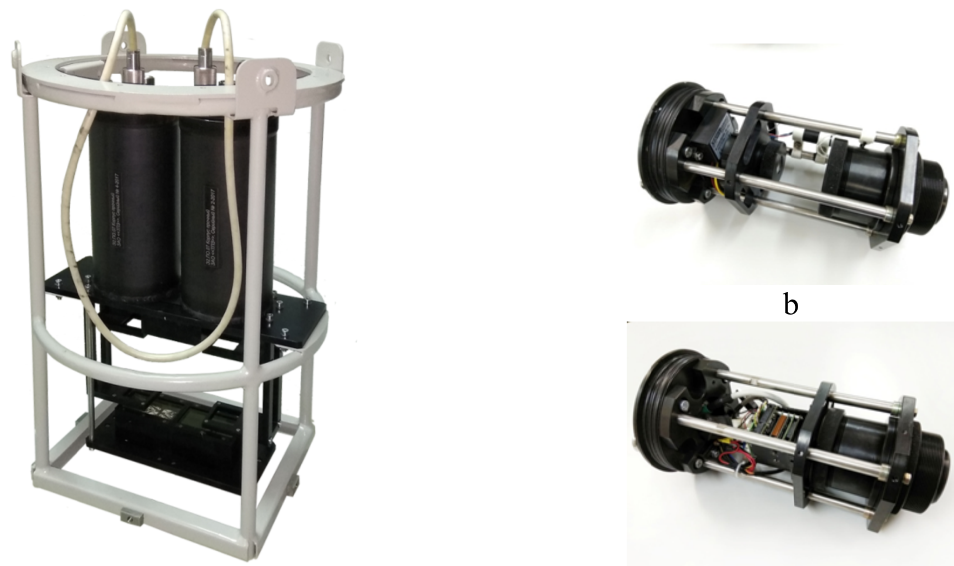


Figure 1. In-line schemes of recording digital holograms in the investigated volume with linear (a) and folded configurations (b). Here I is lighting module, II is registration module, 1 is semiconductor laser, 2 is collimating/receiving objective, 3 is window, 4 indicates the position of test objects, 5 is CMOS camera, and 6 is prism.

Subsequent mathematical processing is performed on a computer. It includes preliminary hologram processing [6] to eliminate the edge effects and to unify the background, calculation of the diffraction integral for level-by-level restoration of the image of the investigated volume of the medium, refinement of the restored holographic image, and retrieval of the desired information. Thus, the DHC technology allows us to record digital holograms of the investigated volume of the medium with particles, to restore the image of each particle, to determine the spatial distribution of particles in the investigated volume (three-dimensional coordinates of each particle), particle sizes, shapes, speed and direction of motion of each particle, and also to recognize them. All operations listed above are performed using the basic software of the DHC technology and allow virtual 3D image of the volume with investigated particles to be obtained.

Figure 2 shows external view of the folded scheme of the DHC. The main parts of the DHC in this photograph are the laser lighting module and the registration module connected with a synchronization line (a cable). The modules are placed into two rugged deep-water housings with windows and sockets. One of the housing is intended for placing of the lighting module (Fig. 2b) comprising the laser and the optical system forming an optical radiation beam. In the other housing, the registration module (Fig. 2c) is placed comprising the optical system to receive optical radiation, the Smart camera to record digital holograms, the system for control over the operating regimes, and the synchronization system. The housings are optically coupled with the mirror-prism system providing the desired investigated volume for hologram recording. In our case, this volume was about 1 L per exposure. The electrical connection of housings and the DHC connection to other devices and complexes (in particular, to communication lines for information transfer) were performed through tight sockets and the cable for marine application.



b

a c

Figure 2. Assembled digital holographic camera (DHC) (*a*), its lighting module placed outside of the housing (*b*), and its registration module placed outside of the housing (*c*).

The lighting module (Fig. 1b) and the registration module (Fig. 1c) placed into hermetic housings can be interchanged using guiding rails attached to a removable flange. This principle provides reparability, option of reservation under unfavorable field conditions, and possibility of modernization or expansion of the possibilities of the employed DHC.

The design is complemented by a metal welded frame with base surfaces for assembling of the complex based on the interchangeability principle. Optionally, the frame can be manufactured in three modifications: with a linear arrangement of housings with modules (scheme in Fig. 1a) along one axis defined by a rigid bar; with the folded optical axis and the investigated volume of the medium intended for vertical scanning (for motion of plankton and other particles predominantly in the vertical direction); with the folded optical axis and the working volume with freely migrating plankton particles and other particles moving predominantly along the horizontal direction (Fig. 2a).

Figure 3 shows the hologram and restored images of plankton species recorded with the digital holographic camera of the marine information-measuring complex during the Arctic Mission.

The patterns of plankton species themselves recorded by the method of digital holography allow one to estimate visually the situation with plankton at the corresponding depth. However, sizes, shapes, velocities of motion, and numbers of particles of various types that constitute the essence of ecological monitoring of plankton biodiversity in the investigated water areas have the most important practical significance.

Therefore, the important characteristics of the complex are the minimal sizes of investigated particles and the representativeness of the sample being analyzed. In real measurement conditions, there are always interfering factors (extraneous suspended particles, turbulent streams, and instrumental limitations) distorting the results of field holographic experiments. Therefore, it is expedient to analyze the characteristics of the submersible holographic camera based on the results of its testing in the plankton habitat. In this connection, the DHC design envisages the possibility of stationary arrangement of test objects (see item 4 in Fig. 1) at desired distances for calibration and estimation of the DHC resolution under field conditions.

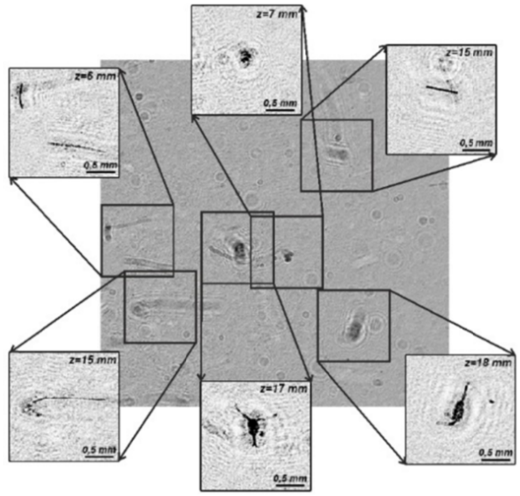


Figure 3. Digital hologram and restored images of plankton particles. The hologram was recorded at a depth of 22.12 m on August 13, 2016 at the stationary station (parking) with coordinates 72º32'49.2" N, 55º30'09.6" E during Mission in the Kara Sea.

3. DESCRIPTION OF TEST OBJECTS AND CONCEPT OF THE RECOGNITION CRITERION

The traditional optical approach to the problem of determining minimal sizes of the displayed (investigated) object is connected with the concept of resolution. To estimate the resolution, the test object used to estimate it should be chosen. The test object should consider the specificity of the object of measurements. Thus, for example, the test of resolution of an optical system using lines of absolute contrast models adequately a one-dimensional periodic signal [7]. However, for the plankton whose particles are low-contrast amplitude-phase three-dimensional objects, it is not suitable by virtue of its one-dimensional character and absolute contrast. Therefore, we used specially prepared test objects [8] most suitable for modeling of plankton particles. An example of such test object is shown in Fig. 4.

Here low-transparent figures of regular polygons, including a hexagon, a square, a rectangle, a triangle, a diamond, and a circle were deposited on the glass surface by the photolithography method using a masking iron oxide layer. The sizes of the figures (the square side, the circle diameter, the sides of the triangle and hexagon, and the short side of the rectangle) were chosen proceeding from the problem being solved; in this case, they were set equal to 200 µm in accordance with the minimal sizes of mesoplankton particles (having sizes in the range 0.2–2 mm [9]).

For successful recognition and classification of plankton species and other particles, it is necessary to have sufficiently contrast boundaries of their images and to recognize their shapes. To estimate the degree of sharpness of the particle image including determination of the longitudinal particle coordinate based on the maximal image sharpness, some methods (including the Tenengrad method, the method of boundary contrast, the method of boundary brightness jump, and so on) [10–15] were developed. In the present paper, the image boundaries are determined by the method of maximum boundary brightness jump.

To estimate the quality of recognition of the particle shape, we used the recognition criterion equal to the minimal particle size a of plankton species, oil drops, gas bubbles, or model test objects for which the differences between the boundary length $l_{im}(a)$ and the holographic image area $s_{im}(a)$ and the corresponding parameters $l(a)$ and $s(a)$ of the particle itself did not exceed a preset value [8, 15]. To calculate the differences $\Delta s = |s_{im}(a) - s(a)|$ and $\Delta l = |l_{im}(a) - l(a)|$, the holographic particle image was preliminary binarized [6]. These differences determine the areas of regions erroneously included into or excluded from the particle image. We call the ratios $\delta_s(a) = \frac{\Delta s}{s(a)}$ and $\delta_l(a) = \frac{\Delta l}{l(a)}$ the criteria for image shape recognition based on its area and boundary.

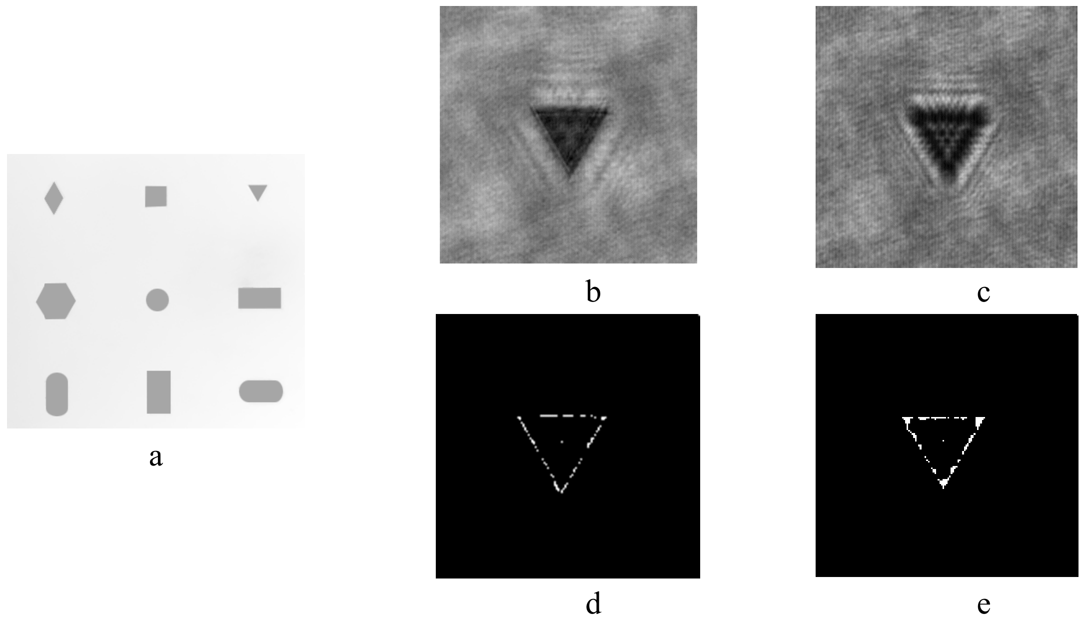


Figure 4. Photograph of the test objects that model particles (a). Holographic images of a triangular particle obtained by the DHC technology in the best focusing plane (b) and in the plane spaced from it by 1.25 mm (c). Binarized images in the corresponding planes (d and e).

For example, in the case shown in Fig. 4, the triangular model particle with side of 200 μm had an area of 1118 pixels and $\delta_s(200) = 0.07$ in the best image focusing plane and $\delta_s(200) = 0.14$ in the plane spaced from it by 1.25 mm. If the recognition criterion is set $\delta_s(200) = 0.2$, both these images can be considered recognizable.

Depending on the problem being solved, other criteria can be chosen, and they can differ by their area and boundary. The a value depends significantly on the noisiness of the scene and the particle shape. From practical experience, it is well known that from the examined model particles, the triangle and the diamond have the worst recognition parameters.

4. EXPERIMENTAL RESULTS

Experiments on recording of test objects in a pool with quiet pure water were performed for two DHC modifications. Figure 5 shows the dependences of the image shape recognition criterion $\delta_s(200)$ on the distance between the windows of the recording DHC module and the test object on the example of the triangular model particle for different DHC configurations. If we set the recognition criterion $\delta_s(200) = 0.2$, we can state that for the linear scheme, the maximal possible recognition distance z is equal to about 600 mm, whereas for the folded scheme, it is equal to about 470 mm. Nearly the same conclusions can be drawn from visual observation of the holographic images shown at the top of Fig. 5.

Analogous dependences, but for the criterion $\delta_l(200) = 0.2$, are shown in Fig. 6. Here the difference between the recognition characteristics is even more noticeable for the two above-considered schemes.

Thus, for the linear scheme, the area recognition criterion is less than 0.2 practically up to 700 mm, and the boundary recognition criterion is generally less than 0.1. At the same time, for the folded scheme, the preset area and boundary recognition criteria (0.2) limit the examined distance up to approximately 470 mm or used to recognize larger particles.

The estimated depths of the holographed medium allow the registered volume of scenes to be estimated for both DHC schemes. As before, we consider that the model triangular particle has a 200 μm side (the minimal size of mesoplankton species) and that the recognition criterion is 0.2. If the beam diameter is 40 mm, the linear scheme allows the particle to be registered practically at all depths of the scene (~620 mm, Fig. 1a), which corresponds to a volume of ~0.8 L.

Under the same conditions, for the folded scheme of the DHC, the admissible depth of the holographed scene is 470 mm and the admissible volume is ~0.6 L.

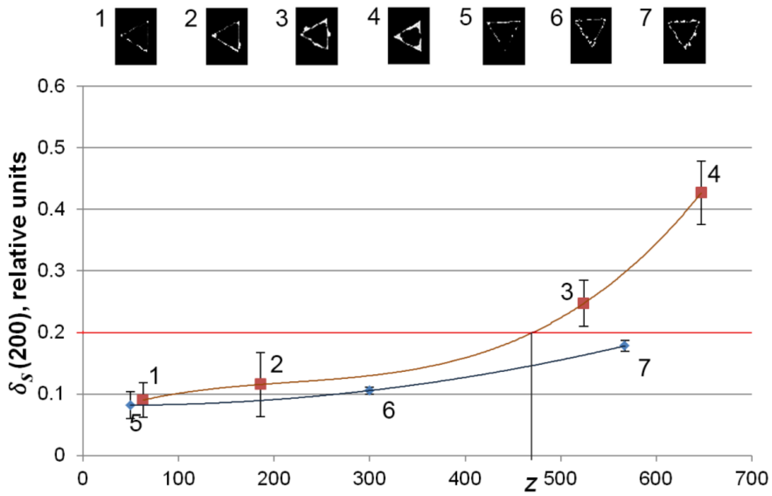


Figure 5. Dependence of the criterion $\delta_S(200)$ for triangular particle shape recognition on the distance between the window of the recording module and the test object for different DHC configurations (orange curve is for the folded scheme, and blue curve is for the linear scheme). Here 1–7 are binarized images of the total areas erroneously included in or excluded from the figure for the corresponding points of the plots. Solid curves show approximation by fourth degree polynomials.

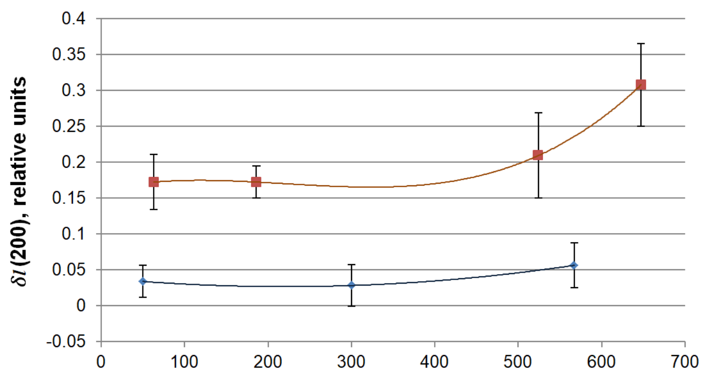


Figure 6. Dependence of the criterion $\delta_l(200)$ of triangular particle image shape recognition on the distance between the window of the recording module and the test object for different DHC configurations (orange curve is for the folded scheme, and blue curve is for the linear scheme). Solid curves show the approximation by fourth degree polynomials.

Such difference is a payment for the reduced mass and dimensions of the folded holography scheme. The application of the reflective prisms significantly complicated the optical scheme. According to the design documentation, the non-flatness tolerance for the executive prism surfaces is ~0.08 µm. The presence of six reflective prism sides caused significant residual wave aberrations that in this case can easily be estimated by summation of random independent errors for each surface; it is equal to 0.45 µm. This value is by a factor of 4.5 worse than the value of the Rayleigh criterion ($\frac{\lambda}{4} \approx 0.1$ µm) for diffraction-limited optical systems to which the linear scheme belongs.

5. OPTIMAL VOLUME AND TIME FOR DHC TECHNOLOGY RESEARCH

The recognition criteria introduced in the previous Section allow us to estimate the volume investigated with the DHC of the corresponding configuration per single exposure when solving a specific problem. This is important for both biodiversity research and ecological monitoring of water areas. Indeed, in both cases it is necessary to provide the possibility of statistical filtration of noise inevitable under field conditions and the representativeness of samples of investigated particles by summation (averaging) of the data for species of different types to retrieve their size and space distributions. Moreover, each subsequent measurement should correspond to the set of particles different from that of the previous measurement. Considering that sizes of the volume registered with the DHC at preset recognition criterion are constant, the representativeness can be provided by the displacement of the measuring volume or by the summation of different temporal plankton realizations. The accumulated experience of DHC application in various missions has shown that measurements performed when the vessel is moving (the measuring volume is displaced) represents a technically complex problem; therefore, it is most expedient to perform summation (averaging) when the vessel is parked stationary by means of successive hologram recording.

In this case, the desired number of holograms can be estimated when the received information reaches the stationary regime, and each subsequent set of values insignificantly (by 5–10%) changes the average result. Here the result (or the obtained information) is taken to mean, for example, the particle size distribution, concentration of particles of various types, etc.

For example, Fig. 7 shows the dependence of the average concentrations of Copepoda, Appendicularia, and Cladocera species, respectively, on the number of holograms used for averaging and their approximations by fourth degree polynomials. The data were obtained during testing of the folded scheme the DHC under field conditions in the Black Sea on the marine stationary platform near the Katsiveli Settlement. The software developed by us for automatic taxon classification of particles with application of the morphological parameter [6] was used. From the plots it can be seen that starting from 35 holograms for Appendicularia and Cladocera taxons, the average concentration remains practically unchanged with further increase in the number of holograms used for averaging, and for the Copepoda taxon, this is observed starting from 20 holograms. Therefore, it is possible to state that in these conditions, the statistically reliable sample for estimation of plankton concentration within the chosen taxons is obtained for averaging of 35 holograms, which for the recognition criteria $\delta_s(200)=0.2$ and $\delta_l(200)=0.2$ set in the previous Section corresponds to the investigated volume of about 21 L.

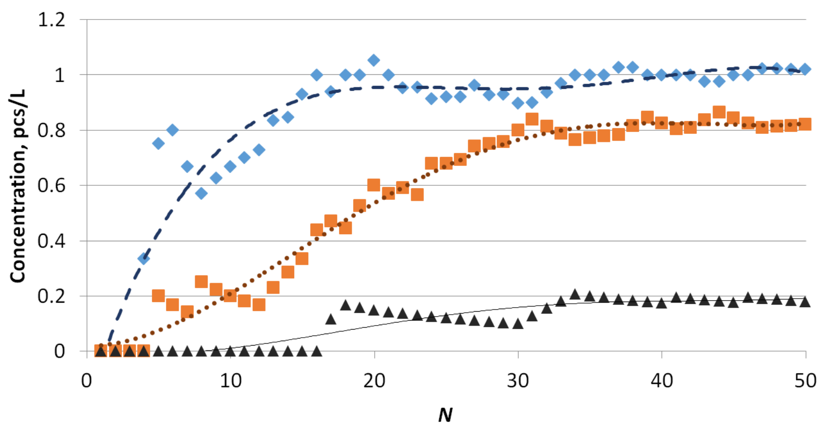


Figure 7. Average value of the concentration of *Copepoda* (blue diamonds), *Appendicularia* (orange squares), and *Cladocera* species (black triangles) depending on the number *N* of holograms used for averaging. Here the dashed, dotted, and solid curves show approximations of the obtained values by fourth degree polynomials.

One of the most important characteristics of the DHC is the rate of hologram processing; moreover, the main goal here is the information retrieval in real time. As a rule, limitation arises caused by the weight and dimensional characteristics, significant computing resources, and performance characteristics of the communication channel. At the same time, it should be noted that the concept of real time should be defined proceeding from the specificity of the object of research and rates of the investigated processes. For example, Fig. 8 shows the results of experiment on DHC application for investigation of diurnal variations of the concentration of plankton species of the indicated types. The data were obtained under field conditions, in the Black Sea, on the marine stationary platform near the Katsiveli Settlement, using the software developed by us for automatic classification of particles over taxons with application of the morphological parameter [6].

From the plots shown in Fig. 8 it can be seen that the diurnal dynamics of the plankton is cyclic in character, with a period of about 10 h. Based on these data, we suggest that digitization period of DHC counts for the parking regime should not be greater than 0.5 h, that is, about 50 counts a day. Thus, in this case the regime of real time is taken to mean that 35 holograms with volume of 0.6 L must be recorded within 0.5 h, which corresponds to the fact that one count averaged over 21 L should be processed.

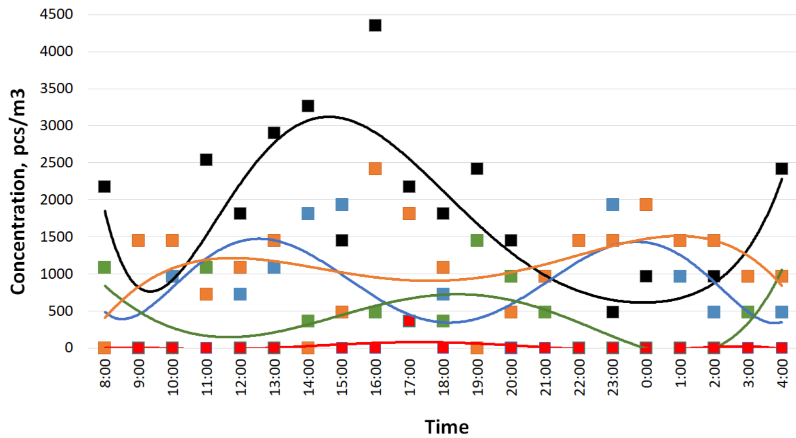


Figure 8. Variations of the concentration of species (*Copepoda*, *Cladocera*, *Copelata*, *Rotifera*, and *Chaetognatha* taxons) caused by diurnal migrations of mesoplankton in the Black Sea (near Katsiveli Settlement) in August, 2018. Here solid curves show approximations by fourth degree polynomials.

6. CONCLUSIONS

In the present work, two modifications of the submersible digital holographic camera (DHC) and their design features have been described. The special realization of the test object and the recognition criteria were suggested for estimation of the capabilities of the DHC technology on recognition and classification of plankton and other particles. It was shown how the assignment of these criteria allowed us to estimate the depth of holographed scenes for the concrete optical DHC scheme and the minimal sizes of the investigated particles, to evaluate the number of holograms that must be recorded to obtain the representative information on the plankton and to estimate the concept of the real time when studying the plankton and other particles.

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