

Article

Study of Marine Particles Using Submersible Digital Holographic Camera during the Arctic Expedition

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Abstract: The paper presents the results of in situ studies of marine particles of different nature using a submersible digital holographic camera (DHC) during the Arctic expedition. It also describes the features, performance specifications, and possibilities of the DHC and the DHC technology. The DHC technology can be used for noninvasive automatic evaluation of spatial and temporal characteristics of plankton, including the distribution of plankton concentrations. The comparison of quantitative analysis of zooplankton net samples and classification results using the DHC revealed that the error of the DHC classification of mesoplankton at the level of the main systematic orders was about 30%. The results of determining the data on the medium, such as water turbidity, according to the radiation shielding factor (degree) by the particles of the *Suspension* taxon using the DHC technology are presented; the prospects for studying the size of gas bubbles and their volume content according to the *Bubble* taxon data are shown. The use of holographic data for in situ point estimates is considered.

Keywords: digital holography; submersible digital holographic camera; marine particles; arctic zooplankton; marine snow; turbidimetry; methane release; floating bubbles

1. Introduction

Marine particles of different origins and nature (sedimentation particles, plankton, gas bubbles, oil droplets, etc.) are one of the most important objects of marine studies [1]. Their study and classification are critical in addressing various tasks: global climate, river-sea interaction, carbon cycle, global warming, biodiversity, and diagnostics of marine ecosystems [2–12], and mathematical modeling of light propagation in seawater [13–15].

Traditionally, marine particles (for example, plankton) are studied through sampling followed by their processing and analysis in the laboratory using a microscope [16,17]. It is obvious that the plankton particles thus studied are removed from the habitat, which makes it impossible to accurately indicate their location in the studied medium. In addition, their state is damaged. Digital holography can be used to solve this problem in situ and in real time [18,19]. In this case, information on suspended particles contained in the fixed seawater volume is recorded as a digital hologram on the CCD/CMOS matrix. The DHC (digital holographic camera) hardware and software complex that we developed for this purpose are described in the works [20,21]. The digital hologram processing algorithms

to extract information on marine plankton are presented in the research by the authors of [22–24]. However, mesoplankton is one of the suspension components, and the DHC can be used to study other particles [25–30].

One of the tasks accomplished during the 82nd Arctic Expedition of the Laboratory of Arctic Research of the V.I. Il'ichov Pacific Oceanological Institute on board the Academician Mstislav Keldysh (AMK-82) from 28 September to 4 November 2020, was to assess the study possibilities and features of such particles.

In addition, in the context of this study, the tasks of the expedition were as follows:

- To determine the equipment's performance characteristics;
- To estimate the DHC measurement error regarding marine particles;
- To assess the validity of a classification.

To address the above tasks, we manufactured the “DHC-probe Arctic”, which includes the DHC and a set of additional sensors that measure the medium parameters (temperature, illumination, pressure, electrical conductivity, oxygen saturation, etc.).

This paper describes the results of using the “DHC-probe Arctic” (hereinafter referred to as the “probe”) to solve these problems. In addition, for the first time, we tried to use submersible holographic data for in situ point estimates of the size and concentration of gas bubbles in areas of massive methane release offshore the Eastern Arctic Seas, which were identified during 2000–2020 [31–34].

2. Methods and Equipment

2.1. DHC Engineering

Holography is used as a noninvasive method that registers a hologram and reconstructs an image of an ensemble of particles. Thus, information on each particle (individual plankton, settling non-living particle, oil droplet, gas bubble, etc.) contained in the registered volume of the aqueous medium can be stored, transmitted, and reproduced at a given time. Figure 1 shows an in-line hologram recording scheme used in the DHC (in-line scheme or Gabor scheme) [35–38].

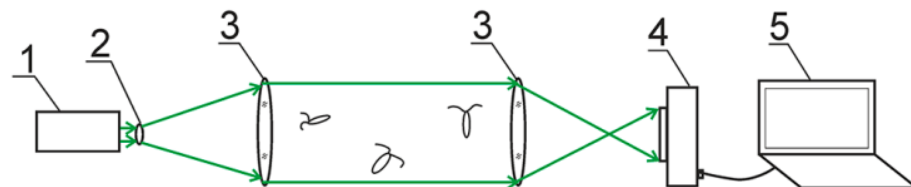


Figure 1. In-line scheme of digital hologram recording: 1—laser diode, 2—beam expander, 3—lens, 4—CCD/CMOS camera, 5—computer.

The interference pattern of the reference wave (radiation that passed by the particles) and the object wave (radiation scattered on the particles) is formed as a result of illuminating the studied volume with laser radiation. The camera registers this interference pattern and transmits it to the computer's memory. The distinctive feature of the DHC is the use of a lens for hologram recording to increase the field of view (the diameter of a beam illuminating the volume of the medium with particles). The lens represents a serial movie projection lens, 35KP-1.8/70 (USSR) with the following characteristics: focal length ($f = 70$ mm), relative aperture ($1/1.8$), and a diameter of the circle of confusion in the field at a wavelength of $0.55\sim 10\ \mu\text{m}$.

Figure 2 shows the 3D model of the DHC horizontal version. The horizontal design implies the vertical scanning of the water column for free flow of plankton in a vertical direction through the working volume of the camera (3). The design provides for four test objects (4) to be placed on the path of the laser beam in the working volume for further magnification calibration. The laser lighting module (2) and the recording module (1) with the CCD/CMOS camera are the main parts of the DHC, which are connected by a synchronization line. These parts are located in two strong, deep-water cases with

portholes and connectors. All other DHC equipment is also placed in these two strong, deep-water sealed cases, which are structurally linked by a frame (6) to the base surfaces for interchangeable assembly. The cases and other devices of the complex are electrically connected by sealed connectors and offshore cables.

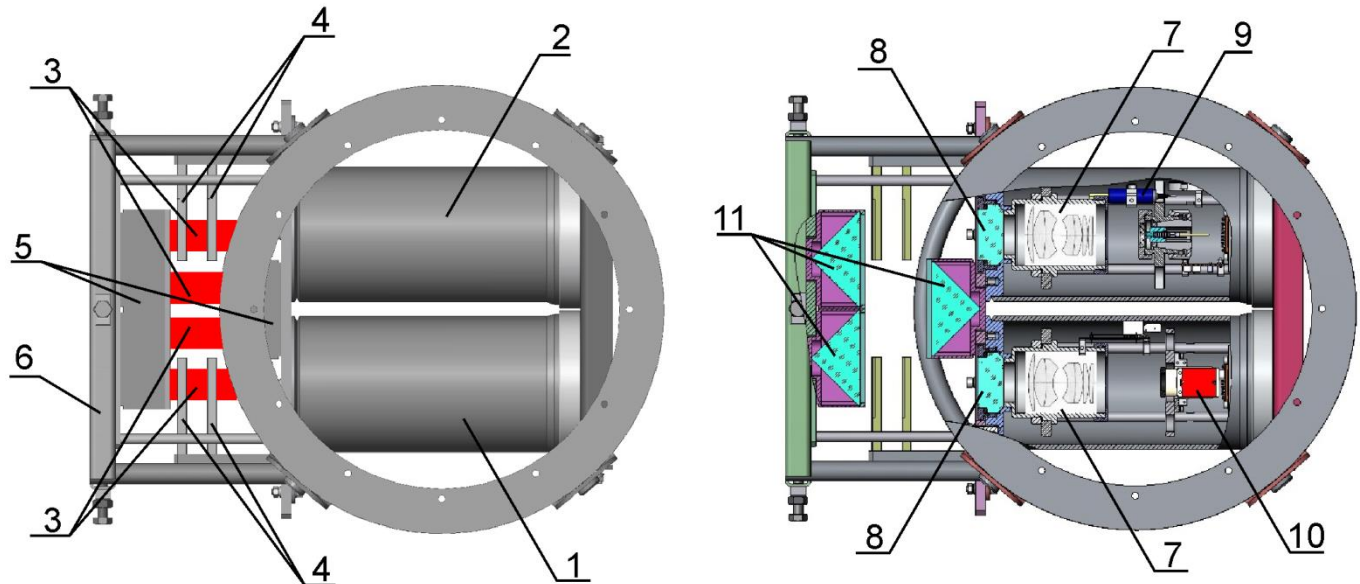


Figure 2. Horizontal version of the DHC: 1—recording module, 2—lighting module, 3—studied medium volume (working volume), 4—calibers (test objects for magnification calibration), 5—mirror-prism system to create a measuring channel in the medium (working volume), 6—submersible device frame, 7—35 KP-1.8/70 lens, 8—portholes, 9—laser-diode fiber module, 10—CMOS camera, 11—measuring channel prisms.

The lighting module (2) (Figure 2) has a laser-diode fiber module (9) and an optical system to create an optical radiation beam (7, 8). The recording module (1) includes an optical system that receives the optical radiation (7, 8), a CCD/CMOS camera (10), and a control system for operating modes (synchronization). The units are optically connected by a mirror-prism system (5, 11) that provides the recording of data in 0.75 L of the studied volume per one exposure.

The design of lighting and recording modules ensures the interchangeability of components (Figure 3). This principle creates the conditions for maintainability and redundancy in unfavorable field conditions as well as upgradability, thus updating or expanding the DHC capabilities or adapting them to various research tasks.

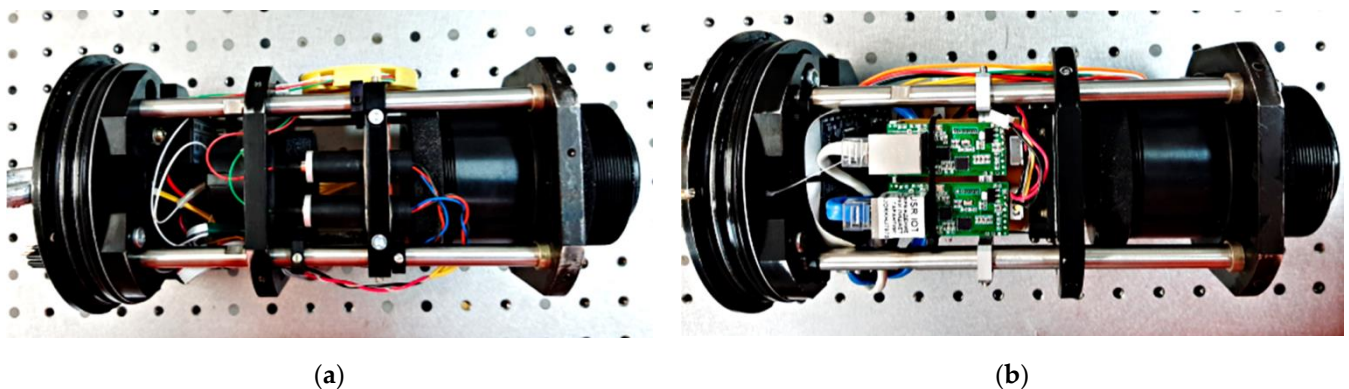


Figure 3. DHC lighting (a) and recording (b) modules outside sealed cases.

Specifications of the DHC with a horizontal submersible device:

- Weight—23 kg;
- Overall dimensions (D × H × W)—581 × 290.5 × 450 mm;
- Variable volume investigated during one exposure—0.2–0.75 L;
- Allowable hydrostatic pressure:
 - Without recalibration—50 A;
 - With built-in calibration—100 A;
- Mako G-507 CMOS camera (manufactured by Allied Vision [39]):
 - Sony DMX264 matrix;
 - Matrix size—2464 (H) × 2056 (V);
 - Pixel size—3.45 μm × 3.45 μm;
- Wavelength of the laser-diode fiber module;
 - For hologram recording—0.66 μm;
 - For illumination and excitation of the phototropic response—0.52 μm;
- Size of measured particles—from 0.1 to 28 mm;
- Sinking speed during vertical probing—0.1–1.0 ppm;
- Discreteness of counts when forming a depth profile—1 m;
- Hologram Ethernet channel speed—1 Gb/s.

Note that the sinking depth of the equipment is determined based on the following points:

- Water depth;
- Limit constraints associated with design features intended for a hydrostatic pressure of 100 A;
- Need to continuously calibrate the increase at depths greater than 500 m due to a significant change in the refractive index of water. In this case, holography is performed with the set calipers (4) (Figure 2).

An information unit of the system represents a set of equipment that performs the functions of communication, calculation, on-board power supply, control, and data switching.

The information unit with a connected DHC makes it possible to install and connect hydrophysical sensors accompanying the plankton measurements. Such an aggregation is traditionally called a probe, and in our case, the hydrobiological “DHC-probe Arctic” (hereinafter referred to as the “probe” (Figure 4)). The design of the DHC probe allows loading it to a depth of 1000 m.

Technical characteristics of the hydrobiological “DHC-probe Arctic”:

- Weight—65 kg;
- Volume studied per one exposure—approximately 0.75 L;
- Permitted hydrostatic pressure—100 A;
- Advantech PCM-9310CQ-S6A1E on-board computer;
- SSD capacity—250 GB;
- Four channels to connect with hydrophysical sensors (RS485 and RS232)—temperature, pressure, microwave conductivity, and CTD (Valeport Mini);
- Communication channels:
 - Wi-Fi backup channel to run and configure the probe for autonomous work;
 - 1 Gb Ethernet (to transfer data on plankton and hydrophysics).

2.2. DHC Software

The principle of building a complex (probe) as an information measuring system assumes the intelligent functionality of the sensor. In the DHC, these functions are fulfilled in the information unit using an on-board single-board computer with the basic DHC software supplemented by software focused on a measurement problem. This separation makes it possible to easily adapt the DHC to a particular task within the framework of one technical solution. The problem-oriented software can have three options:

- To solve operational tasks according to the assessment of the integral characteristics of plankton with a high degree of averaging by volume;
- To solve monitoring tasks related to the classification of plankton individuals and other particles according to preliminary defined criteria, a set of features, and databases of plankton in the studied water area;
- To solve monitoring tasks related to the analysis of plankton behavior by identifying preliminary signs of behavioral responses.

The result of problem-oriented software is a stream of digital data on marine particles. In this regard, the DHC serves as the same data “provider” as other sensors of the information measuring system. The information unit has an SSD drive to store data on board the probe.



Figure 4. A hydrobiological DHC probe in combination with hydrophysical sensors of temperature, conductivity, and pressure on the deck of the Akademian Mstislav Keldysh (1). Operator’s workstation for data acquisition and processing (2).

Problem-oriented software (DHC technology) makes it possible to automatically register a digital hologram (or a sequence of digital holograms) according to specified parameters and then, by mathematical processing, restore the image of each particle according to special computational algorithms. The mathematical processing of a digital hologram makes it possible to obtain images containing information on the distribution of both amplitude and phase of light waves [36,37]. The phase-contrast image of a particle gives information on the volume of the particle, but in order to obtain it, we need to use one of the unwrapping methods [36,37,40,41]. The three-dimensional coordinates of each particle (spatial distribution of particles in the studied volume), dimensions, space orientation, speed, and direction of movement of each particle can be determined within the in situ tasks according

to the amplitude images of particles. Thus, the DHC technology allows for the creation of a virtual 3D image of the volume with the studied particles.

A lens makes the magnification ratio during the reconstruction of holograms dependent on the position of a particle and the refractive index of water. This dependence is described using four coefficients determined during calibration. The magnification ratio taken for different particle positions is one of the functions of the DHC software [42].

The DHC software includes a particle recognition and classification unit that is used to obtain information on individual particles of the medium and their belonging to various taxa. The values of features (decision tree) for automatic classification of particles are presented in Table 1. A rectangle is circumscribed around the reconstructed holographic image of a particle to ensure its automatic classification. Here it is critical to consider the length of this rectangle H and the morphological parameter M , which is the width-to-length ratio of the rectangle, as well as the presence of outgrowths in the zooplankton individual. The values of the relevant criteria for the taxonomy of certain particles are taken on the basis of an experiment, which is one of the tasks of the expedition measurements.

Table 1. Features of taxa for automatic classification of particles: H —length of the rectangle circumscribed around the particle image; M —morphological parameter (width-to-length ratio of the rectangle).

Taxa	Presence of Outgrowths	H , mm	M
1. <i>Chaetognatha</i>	YES	>0.2	0–0.2
2. <i>Copepoda</i>	YES	>0.2	0.2–0.5
3. <i>Appendicularia</i>	YES	>0.2	0.5–0.66
4. <i>Cladocera</i>	YES	>0.2	0.66–0.9
5. <i>Other</i>	YES	>0.2	0.9–1
6. <i>Rotifera</i>	YES	≤0.2	0–0.9
7. <i>Phytoplankton chain</i>	NO	ANY	0–0.25
8. <i>Suspension</i>	NO	≤0.2	0.9–1
9. <i>Marine snow</i>	NO	ANY	0.25–0.9
10. <i>Bubble</i>	NO	>0.2	0.9–1

Taxa 1–7 in Table 1, characterizing mesoplankton, are chosen based on their highest prevalence in the aquatic medium. The *Suspension* taxon is used to determine the turbidity of the medium. It includes particles whose size is less than 0.2 mm, and the section shape is close to a circle, i.e., the height-to-width aspect ratio of the circumscribed rectangle is $M \geq 0.9$. Their size and concentration are used to calculate the turbidity of water. Taxon 9 (*Marine snow*) includes particles that satisfy the definition of marine snow. It includes particles of aggregated phytoplankton, feces, and other detritus components [43–45]. To determine the size and concentration of floating bubbles in areas where methane is released from bottom sediments, the DHC software uses data on the *Bubble* taxon in the studied volume. Here we include data on particles that represent gas bubbles larger than 0.2 mm and whose section shape is close to a circle, i.e., $M \geq 0.9$ (size H for the *Bubble* taxon means the diameter of a gas bubble). Offshore the East Siberian Arctic Seas, bubble methane is released because of the loss of continuity of submarine permafrost, which subsequently creates taliks and destabilizes hydrates [31–34]. Note that the diffusional transfer occurs during the upward movement between the primary methane bubble (in a thin bottom layer) and nitrogen and oxygen dissolved in water, thus increasing the nitrogen and oxygen fractions as the bubble approaches the water-air interface [33,46,47].

The processing results of each hologram are registered as a 2D image with sharp images of particles and a table with data for each particle detected by the algorithm in this hologram. Examples of such data are shown in Figure 5 and Table 2.

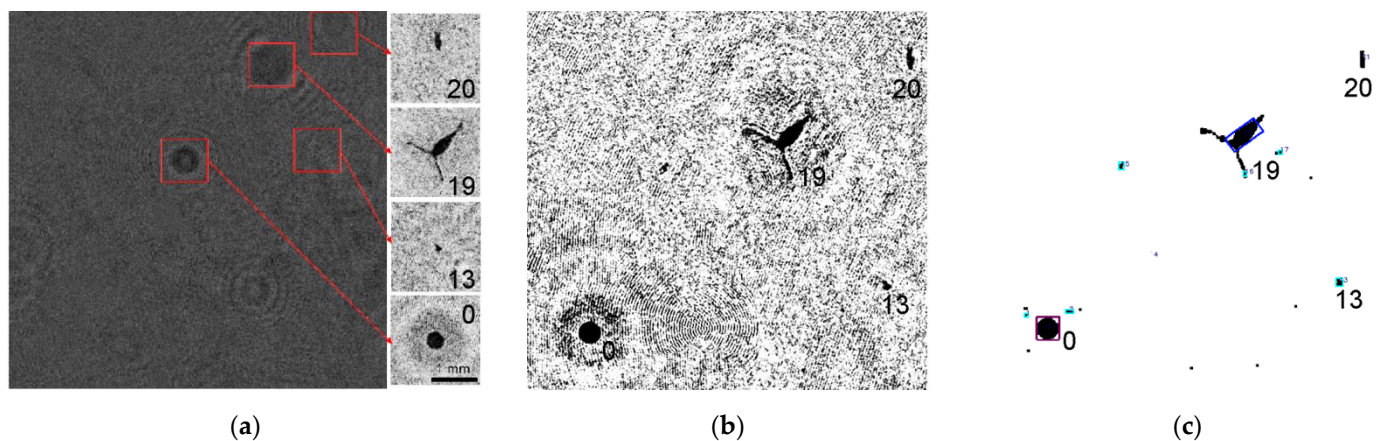


Figure 5. Example of the DHC data after processing in problem-oriented software at the AMK-82 station No. 6939. (a) Digital holograms and particle images reconstructed therefrom. The particle number is the ID number in Table 2. (b) A 2D view of the studied volume. (c) Patterning of the 2D view for particle recognition and classification (the color of the circumscribed rectangle indicates belonging to a particular taxon).

Table 2 shows the degree of completeness and level of detail of the data recorded in a digital holographic experiment in situ.

2.3. Data Analysis

The integral characteristics of clusters of marine particles calculated based on the information contained in the classification tables are used to analyze and compare the DHC data with the measured values accepted in oceanography.

Table 2. Particle data.

ID	Height, mkm	Width, mkm	M	Gravity Center Z, mm	Gravity Center X, mm	Gravity Center Y, mm	Angle, Degrees	Border Length, mm	Particle Square, mm ²	Limb	Depth	Pressure	Temperature	Conductivity	Taxon
0	295.23	295.23	1.00	656.70	6.36	10.85	0.00	1025.72	70,399.07	0	2.57	127174	1	0.011	Bubble
1	52.99	75.70	0.70	411.84	14.26	13.03	0.00	239.64	3552.90	0	2.57	127174	1	0.011	Suspension
2	153.21	100.54	0.66	191.97	13.80	12.36	−18.43	463.38	11,317.72	0	2.57	127174	1	0.011	Suspension
3	45.42	52.99	0.86	602.87	0.70	11.38	−90.00	183.52	2206.24	0	2.57	127174	1	0.011	Suspension
4	211.96	105.98	0.50	269.42	7.44	11.39	0.00	810.84	21,317.42	0	2.57	127174	1	0.011	Marine snow
5	121.12	90.84	0.75	411.84	14.32	11.29	0.00	388.44	7908.08	0	2.57	127174	1	0.011	Suspension
6	52.99	68.13	0.78	347.39	9.37	9.88	0.00	224.50	3209.07	0	2.57	127174	1	0.011	Suspension
7	45.42	45.42	1.00	426.96	13.24	9.83	−90.00	168.38	1919.71	0	2.57	127174	1	0.011	Suspension
8	108.33	213.28	0.51	606.05	10.46	9.42	−63.43	579.31	17,220.12	0	2.57	127174	1	0.011	Suspension
9	136.26	121.12	0.89	608.43	11.07	9.03	0.00	465.98	11,833.46	0	2.57	127174	1	0.011	Suspension
10	68.13	37.85	0.56	322.91	13.12	8.22	−90.00	207.53	2320.85	0	2.57	127174	1	0.011	Suspension
11	52.99	45.42	0.86	202.34	8.85	8.13	0.00	187.95	2005.67	0	2.57	127174	1	0.011	Suspension
12	105.98	68.13	0.64	665.54	10.86	7.82	0.00	317.18	5931.06	0	2.57	127174	1	0.011	Suspension
13	98.41	113.55	0.87	320.65	12.18	5.74	−90.00	409.86	7564.25	0	2.57	127174	1	0.011	Suspension
14	45.42	45.42	1.00	99.05	9.53	5.38	−90.00	177.25	1977.02	0	2.57	127174	1	0.011	Suspension
15	105.98	75.70	0.71	494.98	9.14	4.12	0.00	351.89	4641.70	0	2.57	127174	1	0.011	Suspension
16	45.42	68.13	0.67	147.99	10.86	4.23	−90.00	817.11	13,753.18	0	2.57	127174	1	0.011	Suspension
17	60.56	52.99	0.88	107.62	11.36	3.93	0.00	213.80	2836.59	0	2.57	127174	1	0.011	Suspension
18	45.42	45.42	1.00	509.54	10.37	3.64	−90.00	181.68	2062.98	0	2.57	127174	1	0.011	Suspension
19	83.27	83.27	1.00	379.69	10.28	3.63	0.00	315.34	5845.10	0	2.57	127174	1	0.011	Suspension
20	224.29	494.22	0.45	147.99	10.85	3.69	−36.03	2284.04	97,275.07	1	2.57	127174	1	0.011	Copepoda
21	264.95	90.84	0.34	367.99	12.51	2.63	0.00	705.62	17,965.09	0	2.57	127174	1	0.011	Marine snow

2.3.1. Integral DHC data

The integral data for plankton usually include the concentration (ssp/m^3) and biomass ($1 \text{ mg}/\text{m}^3$) for each taxon. Some literature sources [48,49] provide various methods to determine plankton biomass, including those based on images of plankton individuals [50,51]. The DHC software can be adapted to the requirements of the chosen biomass counting technique. This study determines the maximum size H of the rectangle circumscribed around the reconstructed image of each plankton particle suspended in water, i.e., in its wet state. In this case, the density of a plankton individual of any taxon can be taken as equal to the density of water: $g \approx 1 \text{ mg}/\text{mm}^3$ [52]. To calculate the biomass by wet weight, each plankton particle is represented as an ellipsoid, whose volume is as follows:

$$V = \frac{4\pi abc}{3} = \frac{1}{6}\pi \cdot H^3 \cdot M^2, \quad (1)$$

$$c = \frac{H}{2}, M = \frac{b}{c}, a \approx b = \frac{HM}{2}$$

where a, b, c —semi-axis of the ellipsoid circumscribed around the particle, M —morphological parameter, and H —maximum size of the rectangle circumscribed around the reconstructed image of a plankton particle. The total biomass of k taxon particles by wet weight will be as follows:

$$\text{Biomass}_k = \frac{\sum_{i=0}^n WW_i^k}{V_0}, \quad (2)$$

where $WW_i^k = gV_i^k = \frac{1}{6}\pi \cdot H_i^{k3} \cdot M_i^{k2}$ —wet weight (mg) of the i particle of the k taxon in the DHC measured volume— V_0 . The total biomass of all particles is the sum of the biomass of planktonic individuals of certain taxa.

Turbidimetric and sedimentological measurements are typically performed to clarify the hydrological state of the water area (for example, turbidity measurements). According to the DHC data, turbidity can also be estimated by making a histogram of the *Suspension* particle size distribution (Figure 6).

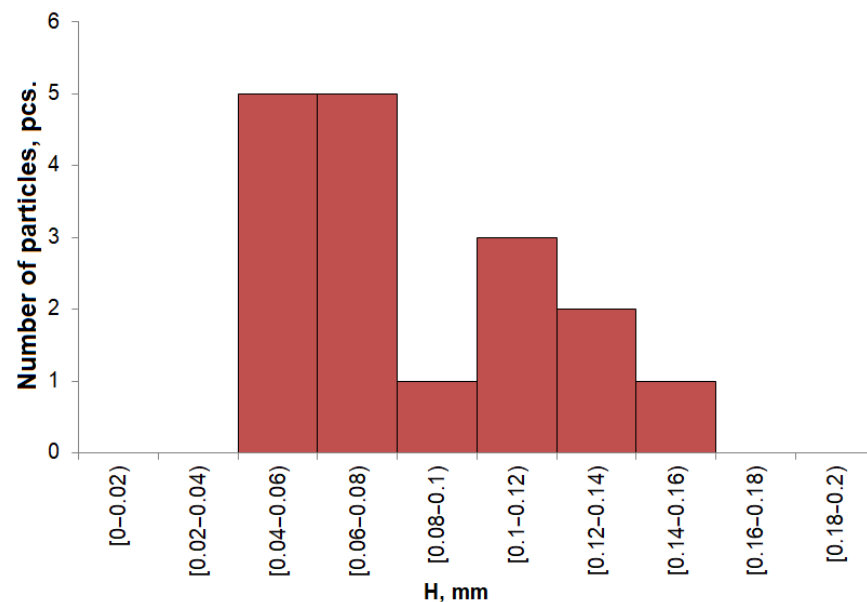


Figure 6. Example of a histogram of the *Suspension* particle size distribution at AMK-82 station No. 6939.

Turbidity is taken equal to the total fraction of the volume section area covered by the sections of the *Suspension* particles:

$$\alpha = \frac{\sum_{i=0}^n S_i}{S_{DHC}}, \quad (3)$$

where S_{DHC} —area of the DHC entrance pupil, S_i —area of the image of the i -particle section, and n —number of particles of the *Suspension* taxon.

Data on the size and number of particles in the *Bubble* taxon are used to determine the volume content of methane. A laboratory bubble test was performed to verify the correct bubble size. The test used square test particles with a size of $500 \times 500 \mu\text{m}$ for calibration (test objects in Figure 2). The Hailea ACO-5501 tank compressor was used as the bubble generator during the test. Capacity: 80 L per hour. Outlet pressure: 0.10 bar. The Hailea ACO-5501 is designed to ensure the effective aeration of water tanks.

Digital holograms of bubbles were registered against the background of test particles. A 2D display of holographic images of the water volume with bubbles was obtained from the focused images of particles and bubbles reconstructed from digital holograms using the DHC technology (Figure 7).

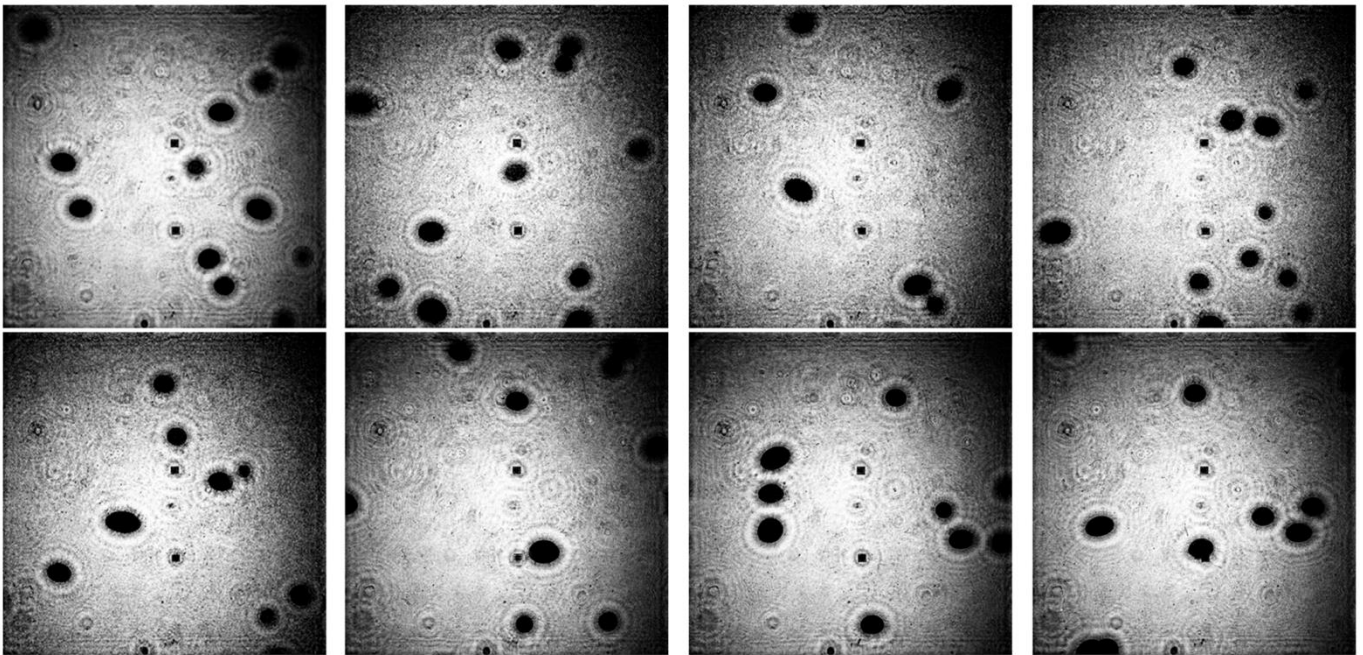


Figure 7. A 2D display of holographic images of the water volume with bubbles.

The size of the bubbles studied in the laboratory experiment was established by comparing the size of the bubble images with the known size of the test particles (model particles), and a histogram of the distribution along the bubble diameter was compiled (Figure 8).

The formula for calculating the volume density of the *Bubble* taxon is used to calculate the volume content of bubbles:

$$V = \frac{\sum_{i=0}^n \frac{1}{6} \pi H_i^3}{V_{DHC}}, \quad (4)$$

where V_{DHC} —studied volume by the DHC, H_i —size of the i particle (bubble) of the *Bubble* taxon, and n —number of particles of the *Bubble* taxon.

The gas flow delivered by the bubble, estimated at a given depth of the probe position, can be calculated using the following formula:

$$p = \frac{\sum_{i=0}^n \frac{1}{6} \pi H_i^3 \cdot v_i}{V_{DHC}} S, \quad (5)$$

where $v_i = \frac{g \cdot H_i^2}{18 \nu_s}$ —ascending speed of i bubble [53], $S = 1 \text{ m}^2$ —sea water surface area accepted for analysis, $\nu_s = 1.787 \cdot 10^{-6} \text{ m}^2/\text{s}$ —sea water kinematic viscosity for 0°C temperature [54], and $g = 9.8 \text{ m/s}^2$ —gravity acceleration. It should be noted that the conversion of the bubble gas flow (a mixture of methane, nitrogen, and oxygen) into methane requires independent studies of the composition of bubbles coming from various types of bottom sediments in the Eastern Arctic Seas, as well as the study of the methane fraction dynamics in floating bubbles of different sizes under various hydrological conditions [47].

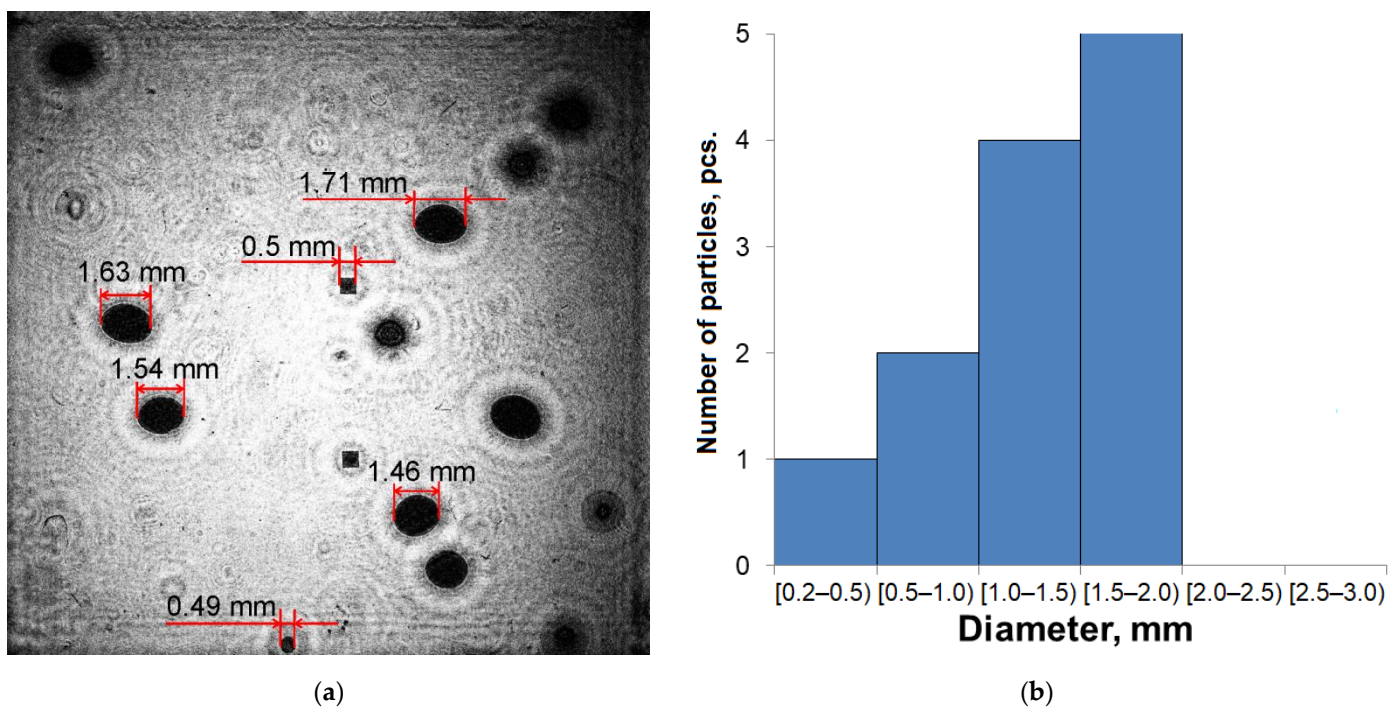


Figure 8. (a) Two-dimensional display of a holographic image of the studied volume with bubbles against the background of the test particles; (b) histogram of the distribution along the diameters of the bubbles.

2.3.2. Methods and Equipment for Verification and Comparison

Data for verification and comparison are obtained by standard methods, namely:

- For plankton—by vertical net catching followed by sample fixation, taxonomic determination, counting of individuals, and laboratory measurement under a microscope;
- For suspension—by processing turbidimetric turbidity measurements;
- For floating bubbles—by using hydroacoustic data of seeps—places of massive release of bubble methane—previously discovered in the Eastern Arctic Seas [31,33,47,55].

Net Sampling of Plankton

Zooplankton was collected by vertical net sampling in the water column using the Juday net with a mesh size of $180 \mu\text{m}$ and a net inlet diameter of 0.37 m . Zooplankton samples were concentrated and fixed with a neutral 4% formaldehyde solution. Subsequently, Rose Bengal dye-stained samples were analyzed in the Bogorov chamber under a stereomicroscope. The organisms were identified according to types, classes, and orders using determinants [56,57].

Turbidimetric Measurements

This is a standard integral method used to determine the turbidity on a study scale of about 1 cubic meter by measuring the scattering of light passed through the water column. The Sea-Bird Scientific USA CTD probe SBE 911plus was used for these purposes and was additionally equipped with a turbidity sensor by WETLabs ECO NTU. The comparison of turbidity versus suspended matter concentrations measured by the weight method shows that the sensor data most likely reflects the real long-term variability of the suspended matter content in the Eastern Arctic Seas.

Acoustic Survey

This is an integral method that uses standard or specialized echosounders and sonars. This method, which is based on the generation of an acoustic pulse and the reception of a scattered acoustic signal, is the most effective for observing floating bubbles and obtaining estimates on the amount of methane delivered by them to the water and surface layers of the atmosphere (Figure 9). Modern hydroacoustic tools are able to detect single floating bubbles at distances of up to 2 km or more, estimate their size, and track their ascending speed [12,47,55,58–64].

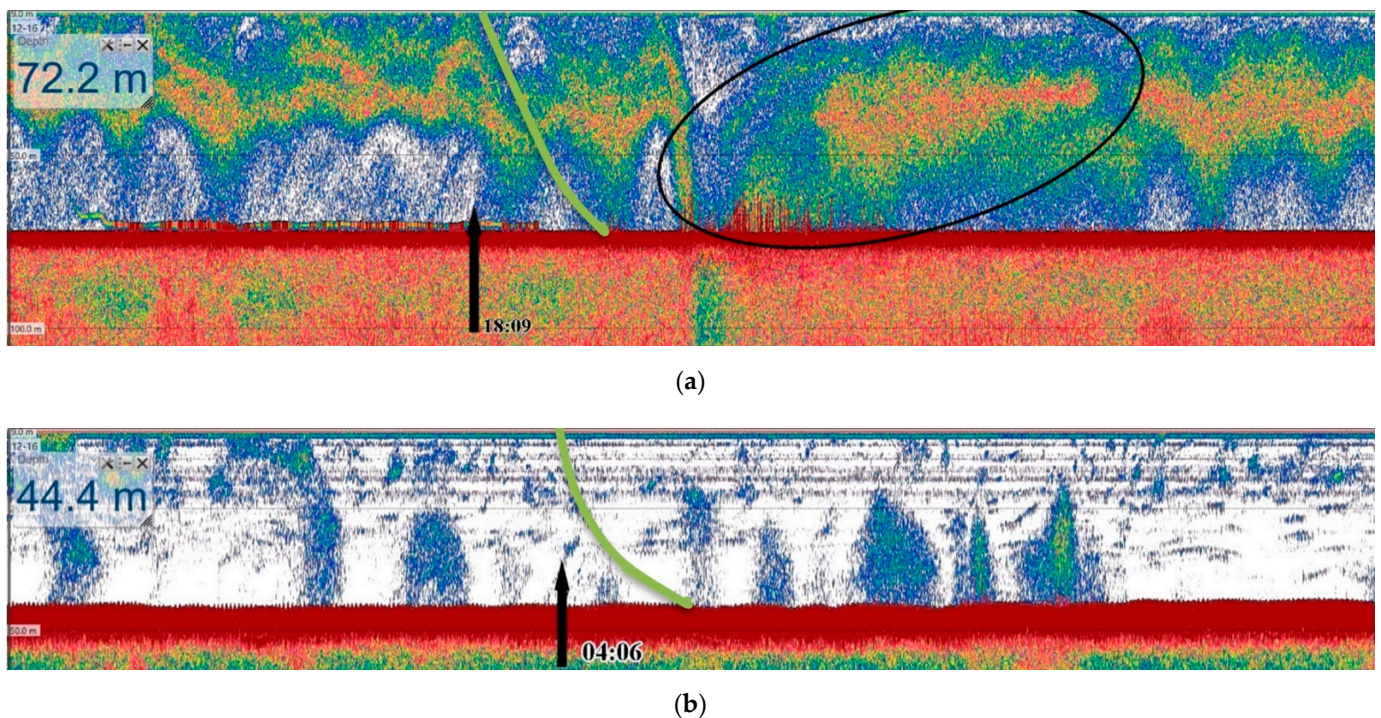


Figure 9. Echograms of drift were obtained at stations (a) No. 6947 and (b) No. 6962. An arrow marks the start time of the hologram recording. An oval indicates a section of the water column with a significant number of bubbles. A green line shows the estimated trajectory of the camera as it drifts.

The experiment in places marked with an arrow was carried out by simultaneously holographing gas bubbles using the DHC. The comparison of the DHC and acoustic data for methane bubbles is not presented in this paper, since the issue of synchronizing acoustic and holographic data on this expedition could not be addressed. Some preliminary results from the expedition are discussed below.

3. Results and Discussion

The classification features of the algorithm were sufficient to explicitly identify the terrigenous suspension, mesoplankton, zooplankton, marine snow, and floating methane bubbles released from the bottom sediments of the Eastern Arctic Seas. We compared the corresponding DHC results with independent data obtained under the same conditions

by other research teams during the AMK-82 expedition to assess the correctness of the processing algorithm (and therefore the choice of the pre-set values of the H and M criteria).

The objects of interest shown in Table 3 were described depending on the geographical location of the stations. Furthermore, the data are given in accordance with these descriptions. A map of the stations considered in this work is shown in Figure 10.

Table 3. Description of the AMK-82 stations.

Station No.	Coordinates	Geographical Description	Depth, m	Range of Interest
6932	72°57′34.8″ N 73°10′13.2″ E	Kara Sea	29	Comparison of turbidimetric data
6941	77°06′07.2″ N 125°05′43.2″ E	Laptev Sea	364	Comparison of turbidimetric data
6947	76°46′33.0″ N 125°49′40.8″ E	Laptev Sea	72	In situ study of bubbles by depth
6961	74°59′31.8″ N 160°58′47.4″ E	East Siberian Sea	45.5	Validation of classification, comparison of turbidimetric data
6962	74°59′25.2″ N 160°59′10.8″ E	East Siberian Sea	45.5	In situ study of bubbles by depth
6975	72°28′57.6″ N 130°32′16.8″ E	Laptev Sea	14.5	In situ study of bubbles in the surface layer of the area of massive methane release
6995	77°54′00.0″ N 105°03′05.4″ E	Vilkitsky Strait	223	Validation of classification

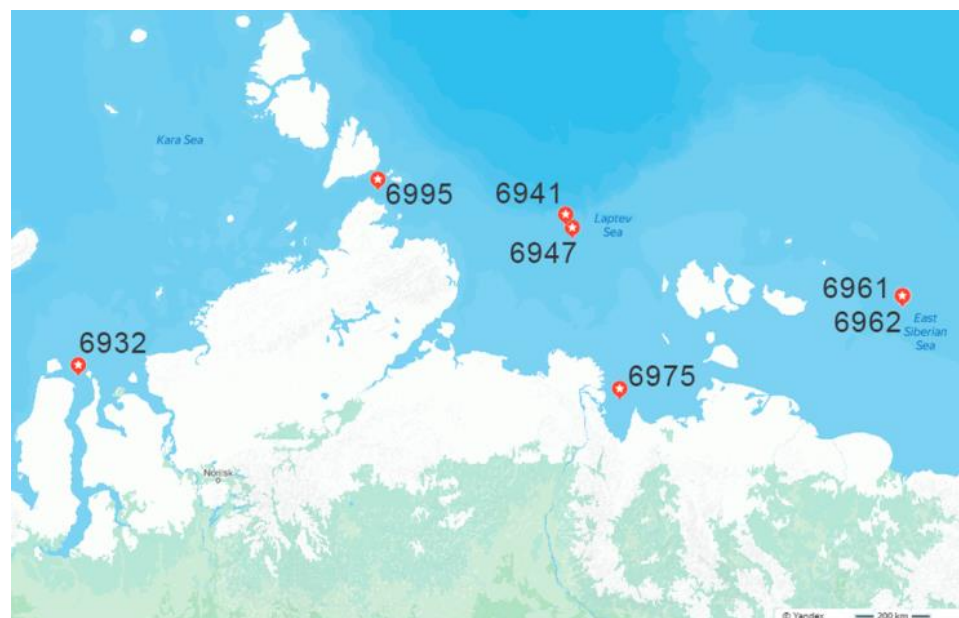


Figure 10. Map of the studied AMK-82 stations.

The operating characteristics of the DHC probe at the stations are given in Table 4.

If the performance characteristics of the DHC are evaluated by the station No. 6961 with the largest number of holograms, then the performance characteristics per 1 m of profile are as follows:

- Time for recording and reading the holographic sample data~3.5 min/m;
- Required memory, taking into account the post-processing data~400 MB/m;
- Data processing time~1.2 h/m.

Figure 11 shows the results of the DHC's performance on the registration of changes in the depth of concentrations of *Mesoplankton*, *Marine snow*, and *Suspension* taxa at two stations. The comparison of the graphs shows that:

- The measurements in the Kara Sea were taken near the Ob River estuary, which showed a very high content of the river-borne terrigenous suspension [65]. These factors determine the high turbidity of the water area and the reduced content of plankton;
- The water was more transparent, and there was more plankton in the Laptev Sea outside the area of the river flow.

Table 4. General performance of the DHC probe.

Parameter	No. 6932	No. 6941	No. 6947	No. 6961	No. 6962	No. 6975	No. 6995
Submersion depth	20 m	109 m	20 m	45 m	19 m	1 m	5 m
Submersion time	2 min	5 min	2 min	14 min	18 min	30 s	2 min
	35 s		40 s	50 s	18 s		44 s
Lifting time	1 min	5 min	2 min	14 min	17 min	30 s	2 min
		20 s	40 s	40 s	18 s		44 s
Wi-Fi read time	11 min	19 min	21 min	120 min	5 min	2 min	14 min
Number of registered holograms	128	231	256	1470	62	28	173
Required memory considering the post-processing data	1.5 GB	2.8 GB	3.1 GB	17.6 GB	0.7 GB	0.3 GB	2.1 GB
Station processing time	4.3 h	7.7 h	8.5 h	49 h	2.1 h	1 h	5.8 h

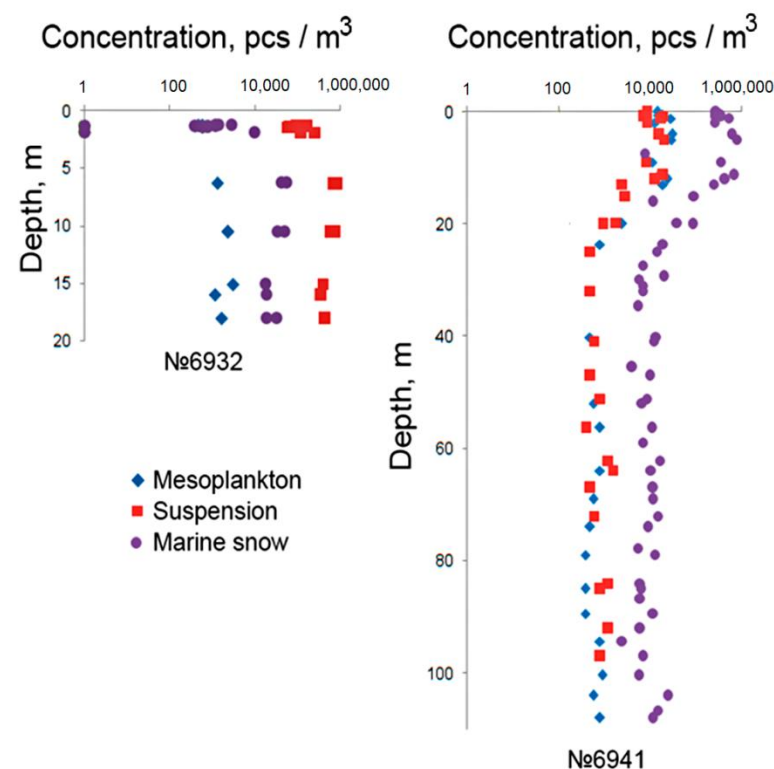


Figure 11. Distribution of *Mesoplankton*, *Suspension*, and *Marine snow* taxa concentrations at depth in various water areas of the Russian Arctic: No. 6932—estuary of the Ob River; No. 6941—Laptev Sea near pack ice. The abscissa scale (concentration) is indicated on a logarithmic scale for simplicity of representation.

Tables 5 and 6 compare the results of plankton number measurements by sampling and analysis with the DHC measurements of plankton.

The analysis of these tables shows that, along with some differences, the classification performed by different methods matches in most cases. Below are the most probable sources of mismatch related to the peculiarities of selection:

1. Different submergence depths of the net and the DHC, different averaging volumes, and different sea states affect the camera shooting quality, as illustrated in Figure 12;
2. The blur of the shape parameter of the *Others* taxon. The mesh size of the net here is 180 μm . For the DHC, the minimum size is $H = 200 \mu\text{m}$ adopted in the classification algorithm (Table 1). Unlike other taxa, this group includes organisms of various shapes. In addition, particles of non-living matter may also belong here.

Table 5. Data for comparison of plankton measurements at station No. 6995.

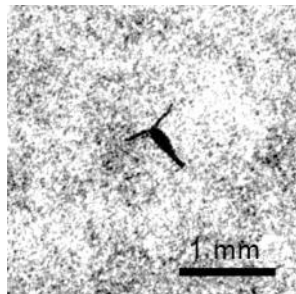
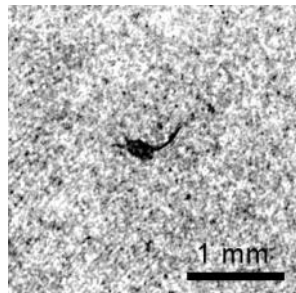
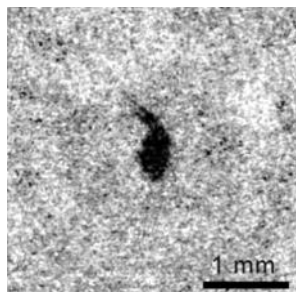
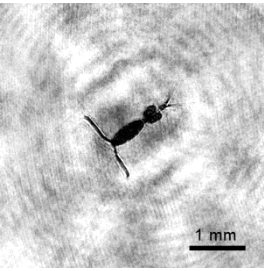
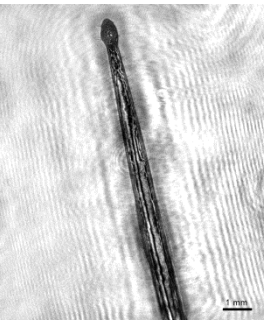
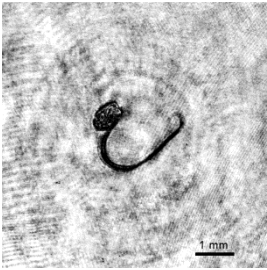
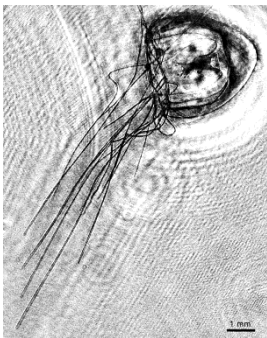
Traditional Classification of Plankton Samples Collected by the Net (Sampling from a Depth of 32 m, Averaging over 3.2 m³)					Classification of Plankton Using the DHC (to a Depth of up to 5 m, Averaging over 0.044 m³)			
Organism, Type	Class	Taxon or Group	Genus	Number, pcs/m³	Taxon	Number, pcs/m³	Holographic Image	
Arthropoda	Crustacea	Copepoda	<i>Oitona</i>	296.9	<i>Copepoda</i>	666.5		
			<i>Calanus/Pseudocalanus</i>	343.8				
		Malacostraca		0.3				
			Chaetognatha					13.1
Chordata	Appendicularia			0.9	<i>Appendicularia</i>	156.8		
Cnidaria	Hydrozoa			1.3	<i>Others</i>	352.0		
Mollusca	Pteropoda	<i>Limacina</i>		78.1				
Others	Others	Larvae		71.9				

Table 6. Data for comparison of plankton measurements at station No. 6961.

Traditional Classification of Plankton Samples Collected by the Net (Sampling from a Depth of 30 m, Averaging over 3 m ³)					Classification of Plankton Using the DHC (to a Depth of up to 45 m, Averaging over 0.825 m ³)		Expert Classification of Holographic Images (to a Depth of up to 45 m, Averaging over 0.825 m ³)	
Organism, Type	Class	Taxon or Group	Genus	Number, pcs/m ³	Taxon	Number, pcs/m ³	Holographic Image	Number, pcs/m ³
Arthropoda	Crustacea	Copepoda	<i>Oitona</i>	400.0	<i>Copepoda</i>	809.0		694.4
			<i>Calanus/Pseudocalanus</i>	500.0				
		Malacostraca		0.0				
Chaetognatha				2.7	<i>Chaetognatha</i>	70		99.2
Chordata	Appendicularia			20	<i>Appendicularia</i>	278.3		199.2
Cnidaria	Hydrozoa			2.3	Others	582.5		496.0
Mollusca	Pteropoda	<i>Limacina</i>		0				
Others	Others	Larvae		3.3				

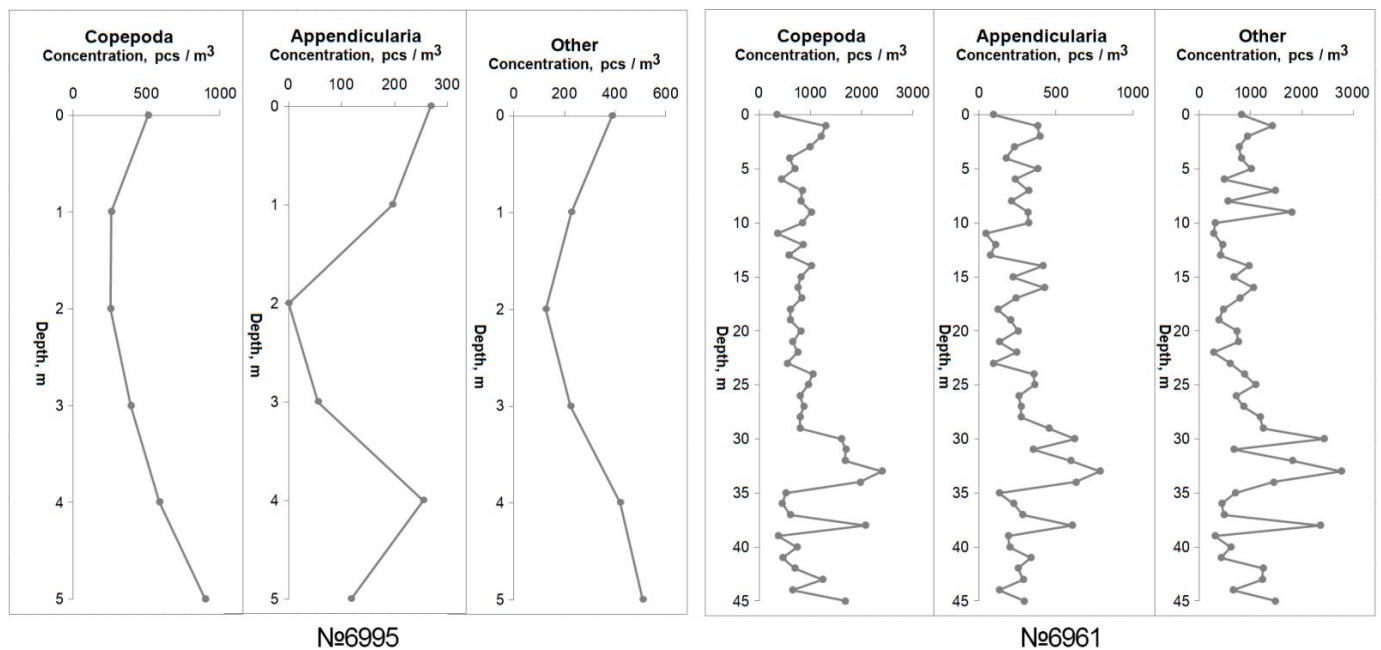


Figure 12. Results of automatic DHC classification of plankton at stations No. 6965 and No. 6961. Depth profiles of *Copepoda*, *Appendicularia*, and *Others* taxa concentrations are given.

Due to these reasons, the DHC performance error is more accurately established through the comparison of data from the automatic and expert classification of images of plankton individuals reconstructed from holograms. For station No. 6961 (Table 6), this single variation does not exceed 30%, depending on the taxon.

Figure 13 shows the comparison of turbidity measurements using the DHC technology and standard turbidimeter data. A good correlation in the data at a significance level of 0.05 indicates the high potential of digital holography for turbidimetry purposes. The correlation coefficient between turbidity measurements using the turbidimeter and the DHC was 75.5%.

Data on methane bubbles away from gas flames in the East Siberian Sea at station No. 6962 and in the Laptev Sea at station No. 6947 are not presented in this paper, since the issue of synchronizing acoustic and holographic data on this expedition could not be addressed. The estimated submergence trajectory of the digital holographic camera shown in Figure 9 by the green line is not certain due to the drift in relation to the research vessel. Highly scattered holographic survey data should be averaged not over five samples at one point taken adrift of the vessel, but over a large area $\sim 1 \text{ m}^2$ of the water surface along the vessel journey. The number of samples in this case increases to 1000. Then the holographic flow measurements will be more representative.

The reverse acoustic dispersion profiles shown in Figure 9 confirm that the holographic results are applicable only for in situ point measurements performed at different distances from gas jets (probe position is marked by a black arrow) and, so far, cannot be used for a representative estimation of the integral bubble flow.

Figure 14 shows the results of the study conducted in a shallow seep in the Laptev Sea at station No. 6975 in the surface layer.

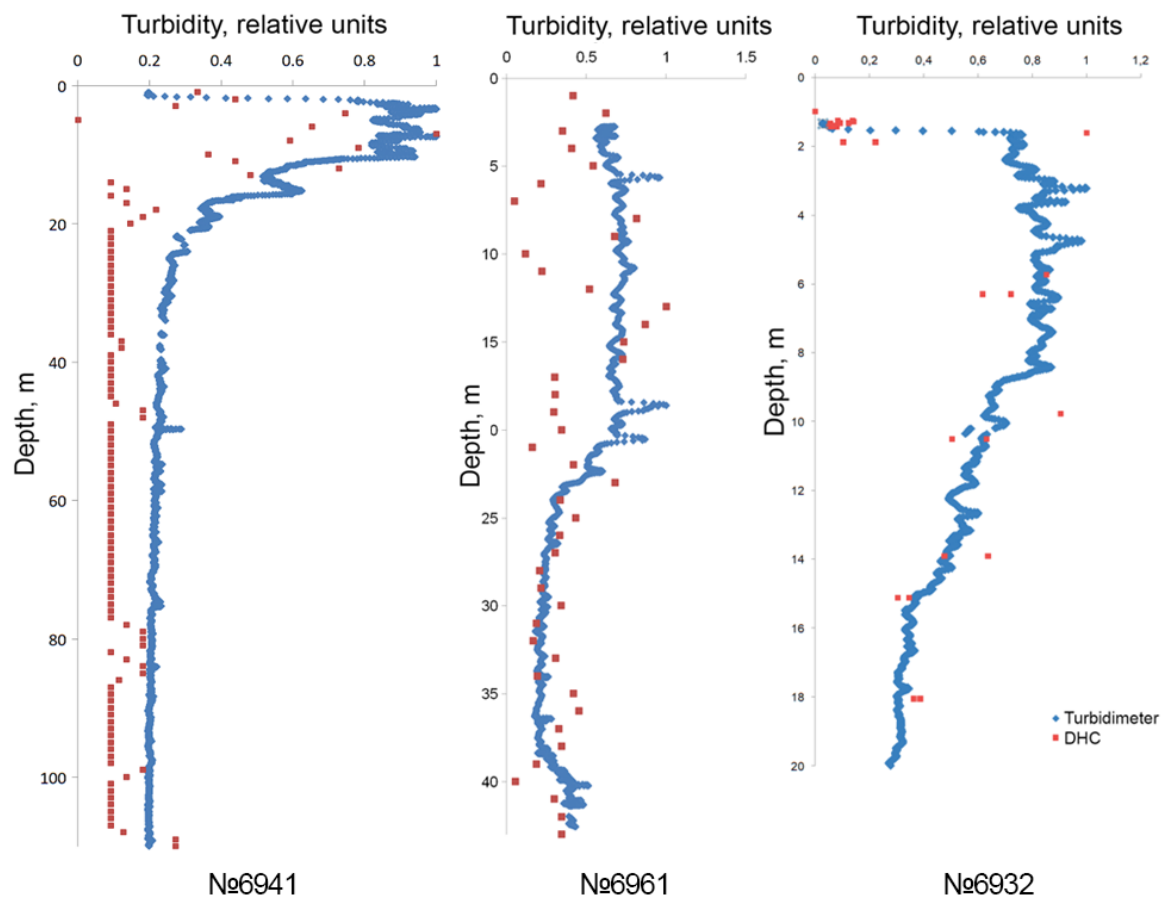
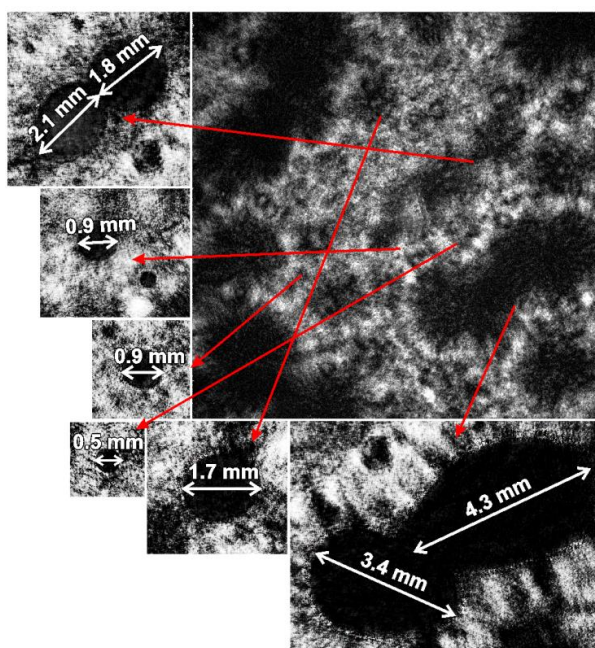
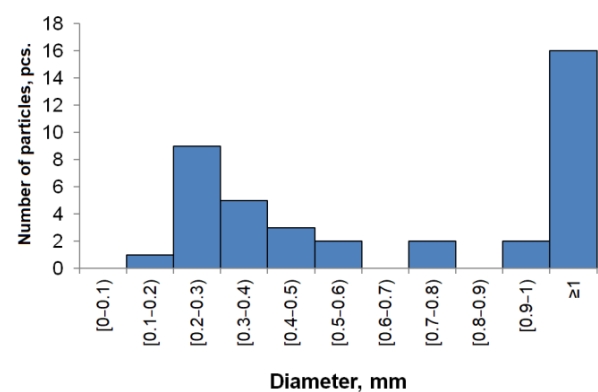


Figure 13. Turbidity results using the DHC technology (red dots) and a standard turbidimeter (blue dots).



(a)



(b)

Figure 14. DHC hologram and some reconstructed images of bubbles in the 1 m surface layer at station No. 6975 in close proximity to the gas flame (a); histogram of distribution by bubble diameters according to the processing of five holograms (b).

At this point, the Academician Mstislav Keldysh research vessel was kept above the seep, which made it possible to obtain new interesting facts on the dynamics of the shape of floating bubbles, including the aggregation effects, which are one of the least studied areas in the field of bubble gas transfer in the bottom sediment-water column-atmosphere system.

Therefore, the holographic probe seems quite promising, provided that further studies are carried out under controlled conditions with a sufficient sample volume.

4. Conclusions

The in situ studies of marine particles during the Arctic expedition using a submersible digital holographic camera (DHC) made it possible to draw the following conclusions on the features and possibilities of the DHC and the DHC technology:

1. The DHC technology in the described configuration has the following performance characteristics (normalized per 1 m of the measured profile in depth):
 - Holographic sampling and recording time~3.5 min/m;
 - Required memory~400 MB/m;
 - Data processing~1 h/m.
2. The DHC technology can be used for noninvasive automatic evaluation of spatial and temporal distributions of plankton concentrations. However, the competent accounting of zooplankton taxonomy at the level of the main systematic orders requires that the signatures serving as the basis for the decision be significantly complicated (compared to a rectangle) and supplemented to achieve errors lower than 30%;
3. The DHC technology can be used to obtain additional information on the medium, namely:
 - Water turbidity estimated according to the radiation shielding factor (degree) by particles of the *Suspension* taxon. Turbidity data obtained using the DHC technology is compared with that measured using a turbidimeter. The correlation coefficient between turbidity measurements using the turbidimeter and the DHC was 75.5%;
4. The DHC technology has certain prospects for its use in the biogeochemical contrast conditions of the East Siberian Arctic Seas. This requires revising the methodology of data selection in the following areas:
 - Holographic survey data shall be averaged along a large area~1 m² of water surface along the path of the vessel;
 - Holographic data shall be strictly bound to an acoustic survey;
 - In-lab DHC vs. generated bubble flux studies.

These conclusions constitute a sufficient basis for the development of a towed holographic probe, which will make it possible to raise complex biogeochemical and methane studies in the East Siberian Arctic Seas to a new level.

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