Digital holography of plankton

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Features of plankton are discussed as a subject for holographic recording. Estimates of possibilities and restrictions are proposed and a large information content of the digital holography of plankton is demonstrated. We present experimental results on digital holographic recording of Baikal zooplankton, including the video on the base of holographic data.

Introduction

Reach information content of holographic methods in experimental studies of spatial ensembles of particles has been demonstrated in many problems.[1–4] The holography makes it possible to register the whole volume of a medium containing the studied particles by a contactless method during a single exposition. In the reconstructed image holography permits one to determine the size, shape, position in the space of every particle, without any preliminary information about the studied volume.

In recent years, holographic methods are actively used in studies of plankton.[4–9] The relevance of such studies is caused by the fact that zooplankton is an important component in food chains of many aquatic dwellers. Ecological situation of an aquatic area can be judged from the state of plankton, which is especially important in regions of anthropogenic impact, in particular, when mining. Finally, the study of the species diversity, behavior, and state of plankton is an individual problem for biologists, limnologists, oceanologists. The above-mentioned unique possibilities of holography point to expedience of their use in solving the listed problems.

The digital holography gives some additional advantages as compared with photo holography. The most significant of them are the following: transmission of holograms by communication lines, what is especially important for submersible holographic cameras; determination of the phase of the reconstructed wave at a given point for the most exact finding of the plane of the best image; making video by holographic data for studying dynamic objects.

Let us enumerate the features of *plankton as an object for holographic recording*.

In real conditions plankton is recorded by submersible holographic cameras. Digital submersible cameras were designed and tested in natural conditions at the University of Aberdeen (Great Britain), Johns Hopkins University (USA), MIT (USA). In Russia, such cameras are still not used because of the absence of corresponding specific scientific programs.

When a submersible camera is used, each plankton particle is holographed through the optical system "water – illuminator glass – air," what leads to aberrations in holographic images, which requires reconstruction.[4,7,8,10] These aberrations can be reduced by using different wavelengths at the stage of hologram recording and holographic image reconstructing,[7,8] as well as by numerical correction.[1] Minimization of the aberrations by the use of a recording scheme with illumination of the studied volume for transmission and normal incidence of the light beam onto the recording plane of the hologram seems to be the most efficient way.[10] Such a scheme can be called the axis scheme with an additional reference beam. Note that digital holograms are also recorded by the axis scheme, although due to another reason: a low resolution (as compared to the holographic photo materials) of CCD- and CMOS-cameras. At the same time, they permit avoiding special measures for the aberration correction, therefore, the problem of aberrations is not considered in this paper.

Plankton particles are living objects, moving in space. In this paper we discuss estimates of the particle motion velocity, pixel sizes, and the time of recording the matrix. Besides, we consider recording of holographic video, which enriches the information content of the holography. In particular, experimental results on determination of plankton particle velocity are presented.

The plankton is characterized by a variety in size and shape: some volume can contain particles of different shapes and sizes, from several micrometers to several millimeters. Therefore, it is necessary to estimate the range of characteristic spatial frequencies of the interference pattern of reference and objective waves, depending on the size range of recorded particles and their details, which are to be resolved. Then the frequencies are to be compared with the pixel size and dimensions of the matrix intended for hologram recording. These estimates are also discussed in this paper.

Plankton particles can be wholly or partially transparent, optically soft. It is well-known that holography allows visualization of such particles. At the same time, the contrast of holographic images of

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such particles can be insufficient to identify them. To improve particle recognition, one can use the algorithm for outlining of the particle holographic image.[11,12]

Moreover, the image, reconstructed from a digital hologram, contains noises caused by discreteness and limitedness of the array during hologram recording. In this paper, the algorithm for reduction of such noises is discussed and its serviceability is illustrated.

It should be noted that layer-by-layer reconstruction of information from a holographic image of a volume of 0.5 m in extent, even at a beam section (coinciding with dimensions of the matrix) of about 25 mm, takes much time, which can reach several hours depending on the algorithm. Therefore, the processing of the information from the plankton hologram (both digital and photo) is a special problem for an individual paper.

Experimental data, presented in this paper, were obtained during two expeditions to the Lake Baikal of the researching group of Institute of Limnology, Siberian Branch of the Russian Academy of Sciences. The first expedition took place in winter, the sampling was carried out at an ice station in 3 km from Ivanovskii cape (near the neutrino telescope); the second one was in summer with the sampling aboard the research vessel *Vereshchagin*. Vertical closing Jedy nets (1/10 m^2, a filtering cell of 90 μm) and JOM, Jedy oceanic model, (1/2 m^2, 160 μm, respectively) were used in the sampling from depths between 0 and 1400 m. Living plankton from the samples was placed into a $4\times4\times3$ cm cell with water, holographed, and then fixed in a 4% formalin solution for further study by standard methods. Totally, about 15 000 plankton holograms were recorded; some of them are used in this paper as illustrations.

Recording and reconstruction of particle digital holograms

Let us briefly remind the main principles of the digital holography. At the stage of hologram recording, the interference pattern of the reference and objective waves is recorded not to a photographic material but to a CCD- or CMOS-camera. As it was mentioned above, digital holograms are recorded, using of the axis scheme (Fig. 1) because a low resolution ability (as compared to holographic photo materials) of the CCD- (CMOS)-receiver.

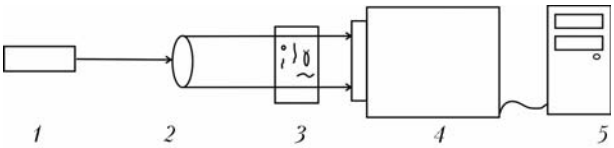

Fig. 1. Axis scheme of recording a digital hologram.

Radiation of the laser *1* is formed by a collimator *2* into a beam of the necessary cross section and illuminates the recorded volume *3*. In our case, this is a water volume containing the studied plankton particles. The radiation, scattered by particles, is the objective wave, and the radiation, passing by the particles, is the reference wave. The interference pattern of these coherent waves is recorded by the digital camera *4* and is stored in the computer *5* as a two-dimensional array of intensities.

This paper presents experiments, in which a He–Ne laser ($\lambda = 633$ nm) and a Videoscan-2020 camera were used.

Consider the requirements to the digital camera. It is evident that the camera must give a sufficiently detailed record of the interference pattern of the reference and objective waves. To estimate the required characteristics of the camera, let us consider a record of an axis hologram for a non-transparent spherical particle of the radius a, placed at the distance z from the recording plane of the hologram. In the case of a diffraction far-field record, the interference pattern of the reference and objective waves is an Airy pattern modulated by a sinusoid.[13] If we suppose, as in some papers, that non-distorted information on the particle shape can be obtained only by recording one central and three lateral maxima of the Airy pattern, then it is easy to estimate the radius of a single particle hologram (it equals $2\lambda z/a$), as well as the period of the interference pattern at the border of the particle hologram ($a/2$).[13]

Based on the fact that the pattern is recorded by discretely placed pixel of finite size, preset an obviously rigorous estimate of the required pixel size as $a/10$ and obtain that, for instance, the Hitachi KP-M1AP camera (matrix of 8.72×6.52 mm, pixel of 11.6×11.2 μm) provides for resolution (record of particles or their details) of 116 μm and higher. The SK-2005 camera (matrix of 3.6×4.8 mm, pixel of 9.8×6.3 μm) provides for resolution of 98 μm. In our experiments we used the Videoscan-2020 camera (a matrix of 11.84×8.88 mm, a pixel of 7.4×7.4 μm, a minimal exposition of $35\cdot10^{-6}$ sec), providing for resolution of 74 μm.

For recording by radiation with $\lambda = 633$ nm, the maximal distance between the particle and the recording plane (depth of the scene), at which the above-stated estimate of the hologram size holds, equals 300, 140, and 260 mm for the above-mentioned particle sizes, respectively.

In our case, the limit velocity of a moving particle is accepted, at which the interference pattern of the objective and reference waves during the exposure time is shifted by 0.1 of its minimal period, i.e., by $a/20$, which is equivalent to a half of a pixel. Then, for the listed cameras with minimal exposure time of about 10^{-5} sec, a particle of a size of 100 μm can be recorded, if its velocity does not exceed 50 cm/sec. This condition is surely satisfied for plankton individuals.

Note that the comparison of the above-mentioned resolution estimates with parameters of, for instance, Baikal zooplankton,[14] demonstrates a possibility to recognize most of plankton individuals by their holographic images.

Finally, it is necessary to take into account the features of the recorded pattern, whose envelope is an Airy pattern, characterized by a wide range of intensities at different ranges. For instance, the magnitude of the third maximum is less than that of the Airy pattern central intensity maximum by a factor of 10^3. This allows one to estimate the word length of the signal digitization, provided by some camera, as not less than 12.

If a CCD- (or a CMOS-) camera satisfies the above-mentioned requirements, one can consider that the recording regime of the digital hologram is ideal, as it is called in traditional holography.[15] In this case, the camera records a two-dimensional array of intensity distribution for the interference pattern of the reference and objective waves, which can be used to a constant as the complex amplitude $u(x_1, y_1)$ of the reconstructed wave in the plane (x_1, y_1) just behind the hologram. Then, to calculate the complex amplitude and the intensity of the reconstructed wave $u(x_2, y_2)$ in any considered plane (x_2, y_2) at a given distance z behind the hologram, the well-known diffraction integral can be used

$$u(x_2,y_2) = \frac{e^{ikz}}{i\lambda z} \times$$
$$\times \int_{-\infty}^{\infty}\int_{-\infty}^{\infty} u(x_1,y_1)\exp\left[\frac{ik}{2z}\left((x_2-x_1)^2+(y_2-y_1)^2\right)\right]dx_1dy_1, \quad (1)$$

where k is the wave number.

This diffraction integral can be calculated by different algorithms, for instance, by the convolution method, which includes the calculation of two direct and one inverse Fourier transforms. We used the program, which is based on the direct calculation of the diffraction integral in order to minimize errors, generated by different approximations.

Preliminary processing of a hologram

In the image, reconstructed from a digital hologram, noises are observed in the form of a system of bands (Fig. 2*c*), caused by the discreteness and the limited data array. If the hologram were reconstructed by the traditional optical way, the considered noises could be interpreted as a diffraction pattern at the hologram border. Then, from the physical point of view, these noises can be reduced either by increasing the hologram size or by smoothing the frame border.

We tried different ways of the hologram supplementing: a frame multiplication on all sides of the hologram, supplementation by frames with mean intensity, etc.[12] The following algorithm proved to be the most efficient: a hologram (Fig. 2*a*) is approximated by bicubic splines and is extrapolated on all sides so that the intensity and its derivative are zero at the borders of the obtained frame (Fig. 2*b*).

Figure 2*c, d* presents images of a plankton particle, reconstructed from the holograms of Fig. 2*a, b*, respectively. It is seen that this algorithm efficiently eliminates noises, caused by the discreteness and boundedness of the array.

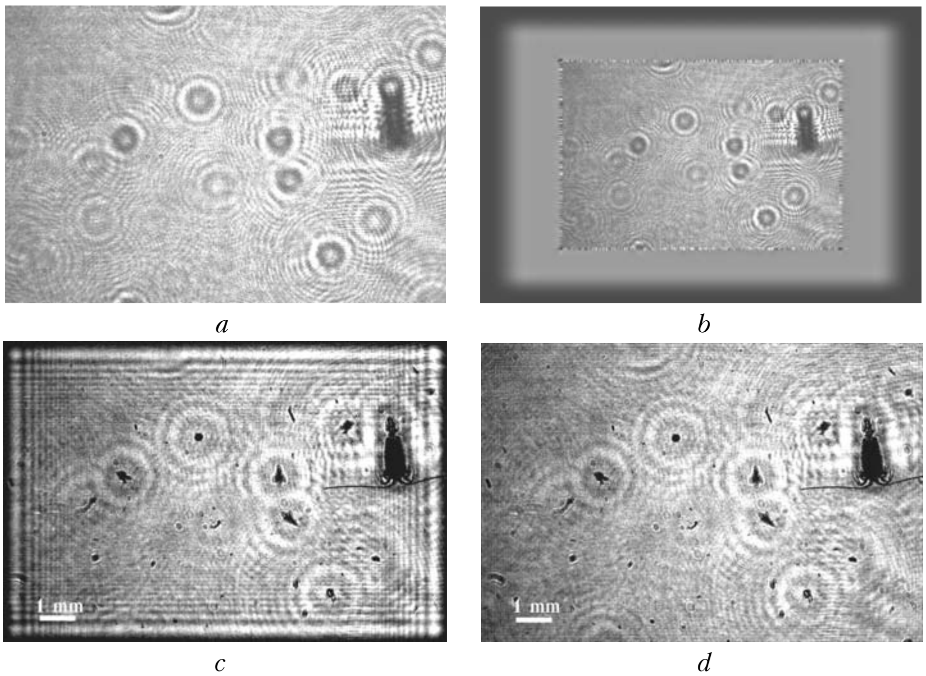

Fig. 2. Elimination of noises in a holographic image of a particle by preprocessing of the hologram: the initial hologram of a plankton particle (*a*); the completed hologram of the plankton particle (*b*); the holographic image of the plankton particle, reconstructed from the initial hologram *a* (*c*); the holographic image of the plankton particle, reconstructed from the completed hologram *b* (*d*).

All holograms in this paper were preprocessed by the described algorithm.

Reconstruction of a single hologram

The process of layer-by-layer reconstruction of an image of a volume with plankton particles is schematically illustrated by Fig. 3*a*. Figures 3*c*–*e* present experimentally obtained images of different planes of the volume.

There are no sharp images of particles in Fig. 3*c*, this distance corresponds to plane *1* (Fig. 3*a*). Varying the reconstruction distance under the integral (1) with a given step Δz, one can find plane *2* (see Fig. 3*a*), where the plankton individual is sharply represented in the top right part of the frame (Fig. 3*d*), and plane *3*, where the plankton individual is sharply represented in the left bottom part of the frame (see Fig. 3*e*).

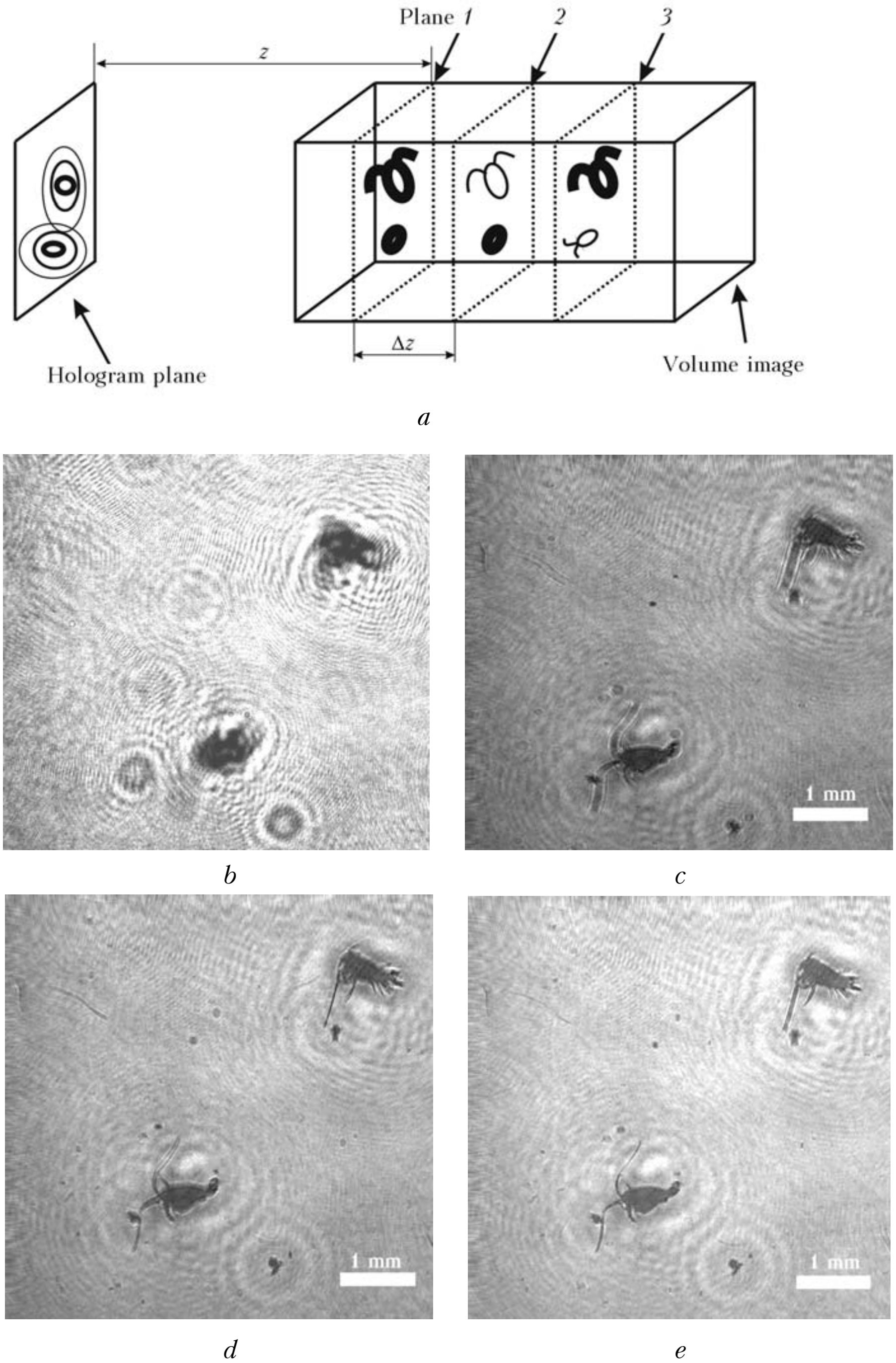

Fig. 3. The process of layer-by-layer reconstruction of the holographic image of a volume containing plankton particles: schematic representation (*a*); digital hologram of plankton (*b*); images reconstructed at different distances from the hologram, mm (*c*, *d*, *e*): *c* – 184 (*c*), 194 (*d*), 199 mm (*e*).

Record and reconstruction of the holographic video

Holographic video is a video film made on the base of holographic data. To do this, the interference pattern of the reference and objective waves is continuously recorded by a CCD- (CMOS)-camera (with the frame repetition rate of the camera) and recorded into a computer as an AVI-file. Then the obtained AVI-file is divided into individual BMP-files (frames). Each of the obtained frames is a hologram, recorded at a known instant and containing the information about the whole studied volume at this instant. Then each single holograms is preprocessed and the holographic image is retrieved from the each hologram.

Note that there are different ways of choosing the image reconstruction plane. For instance, if the plane of the best image is determined for a particular particle at the image reconstruction from each hologram (frame), then this particle is always represented sharply, and there is a possibility to analyze its dynamics during recording the holographic video. If the image reconstruction plane is fixed, one can observe all particles passing through this plane of the volume during the recording time. Then the reconstructed images (frames) are again merged in the video film.

Examples of holographic video can be found in the Internet:

ftp://nfpk:nfpk@video.tsu.ru/geo/plane.avi;
ftp:// nfpk:nfpk@video.tsu.ru/geo/video1.av;
ftp://nfpk:nfpk@video.tsu.ru/geo/video2.avi.

The process of adjusting for sharpness (refocusing) is illustrated in the file ftp://nfpk:nfpk@video.tsu.ru/geo/focusing.avi.

Figure 4 presents a sequence of four holographic video frames for the case when the reconstruction plane is fixed to the focused image of the particle marked by *3*. The particle is seen distinctly in all frames, while the sharpness of other particles varies from frame to frame.

Measurement of plankton particle velocity

It is evident that the holographic video allows supplementing the information about geometric parameters of a particle and its position in the space by information about its velocity and motions in the space. Actually, coordinates of a particle can be determined in each frame of the holographic video, and the time between these frames is defined by characteristics and operation regime of a CCD-camera.

Table presents an example of such measurements for the individual, numbered as *1* (see Fig. 4).

Here the calculations were performed for 14 sequential frames, which correspond to 2.8 sec. (the frame repetition rate in the Videoscan-2020 camera used in the experiments was 5 fr/sec). Mean velocities of particles *1*, *2*, *3* (see Fig. 4), determined during that time, were 3.9, 2.47, and 1.22 mm/sec, respectively.

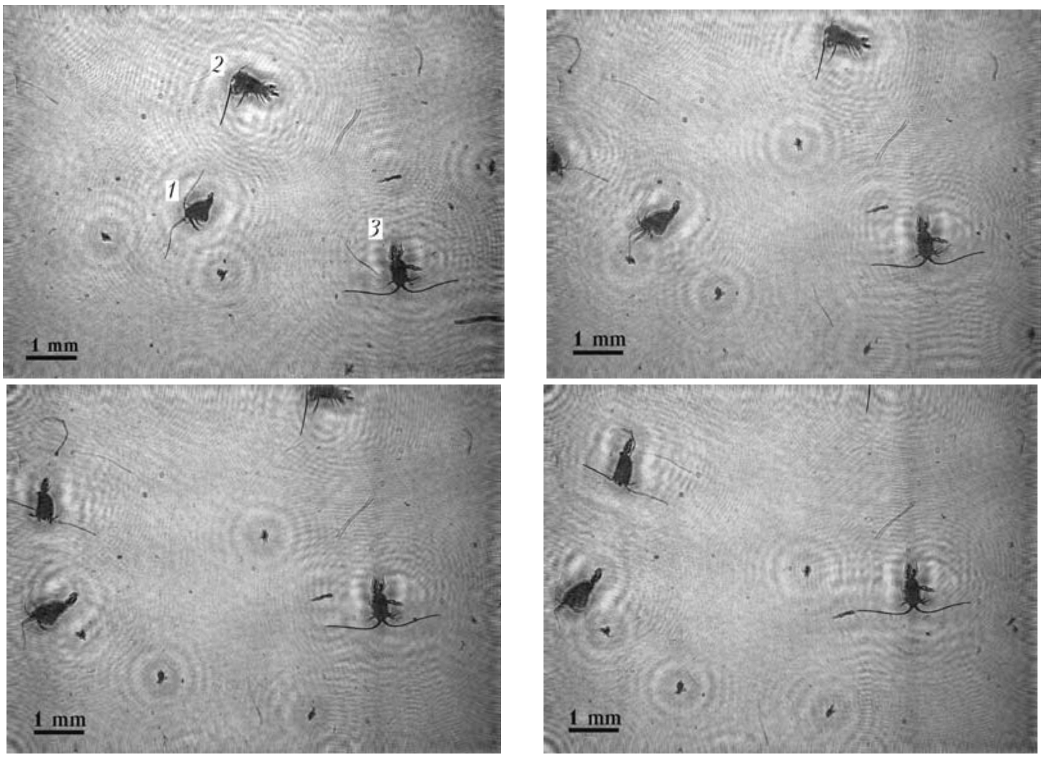

Fig. 4. The sequence of holographic video frames. Particle 3 is always focused.

Frame number	Coordinate, mm			Velocity, mm/sec
	x	y	z	
1	4.57	6.72	194	1.15
2	4.5	6.94	194	6.56
3	3.87	6.39	193	10.74
4	3.09	6.28	191	0.74
5	3.09	6.42	191	0.42
6	3.08	6.51	191	0.82
7	3.07	6.67	191	5.48
8	2.63	6.55	190	7.91
9	1.14	6.03	190	5.09
10	1.03	6.18	189	5.05
11	0.96	6.31	190	0.97
12	0.86	6.48	190	0.7
13	0.86	6.62	190	5.01
14	0.85	6.68	189	1.15

To measure the velocity of particle motion during inter-frame intervals, the plane of the best reconstruction was determined and coordinates of a selected characteristic detail were measured for each hologram (frame). When it was necessary to eliminate the error, connected with rotations of particle, the same volume of the medium was recorded at two camera angles.

Thus, the holographic video permits one to study each particle of a volume both in the space and in time. This is evidently accompanied by the possibility to construct a motion trajectory for each individual of the plankton.

Conclusions

The presented estimates and experimental data demonstrate a rich information content given by the digital holography for study of plankton. Like in photo holography, there is a possibility to obtain information about geometric parameters (size, shape, position in space) of each plankton particle in the studied volume. Besides, the advantages of holographic methods are supplemented by advantages of the digital holography: a possibility to transfer holograms by communication lines, to create holographic videos, and others.

At the same time, traditional photo holography has an advantage in the resolution and the scene depth due to the small size and low (as compared with holographic photo materials) resolution of the present-day CCD-matrices. For instance, in field experiments[7] with pulse recording of holograms on holographic photo plates, a 10 μm resolution of the plankton details was reached at a scene depth of up to 470 mm. In this paper, the resolution was also estimated relative to known dimensions of plankton parts and equaled 90 μm. The scene depth was bounded by the size of the used cell (30 mm), although in the previous experiments we reached a scene depth of 400 mm. It is evident that a higher resolution and a larger scene depth can be reached by cameras with larger matrices and smaller pixel size.

The holographic video gives additional possibilities, which allow one to study dynamics and interaction of particles, measure their velocity, construct trajectories of the particle motion. The permissible velocity of the particle motion is determined by the minimal time of exposure of the camera; the temporal resolution is determined by the repetition rate of frames. In this paper, the Videoscan-2020 camera made it possible to record 5 fr/sec, therefore the temporal resolution turned to be 0.2 sec. The temporal resolution can be increased, when using cameras with a higher rate.

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