Plankton detection using a simulator of a submersible holographic camera

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To study small particles in their natural medium (for example, plankton or particles settling in water), it is reasonable to use methods of pulsed underwater holography, which, due to the sufficient resolution and the scene depth, allow one to obtain, during single exposure, information about every particle of the entire volume of the medium studied. We have designed a setup, called a simulator of a submersible holographic camera, which is an autonomous laboratory model of a full sized camera. The simulator is intended for development of the methods of underwater holographic shooting, improvement of the technologies of assembling the camera, adjusting the optical system, recording holograms, loading a photoplate holder, shot changing, maintaining the laser system, and so on. Results of experiments on recording model particles and plankton particles moving in water are presented. The volume under study was about 1 liter (with the length of 500 mm), the resolution is 200 μm. Problems of entering the holographic images into the computer and their numerical processing in order to improve the quality and distinguish the objects' boundaries are discussed.

Introduction

Recording of the volume ensembles of particles is urgent in various fields of science and technology, for example, in solving such problems as measurement of the particle size and concentration of different aerosols under laboratory and field conditions,[1,2] investigation of two-phase flows,[3] study of the processes of laser radiation interaction with aerosol,[4] and observation of plankton in aqueous media.[5,6] The appropriateness of the holographic methods for these purposes has been justified in Refs. 1–7. The holographic methods do not disturb the volume surveyed, and, having sufficiently high resolution and scene depth, they allow the information about all particles in the volume to be recorded during a single exposure. Moreover, one hologram bears the information both on every individual particle (size, shape) and on the entire ensemble (particle number density, mutual arrangement in space), as well.

The possibility of determining the refractive index of particles having regular shapes (sphere, ellipse, cylinder) from their holographic images has been demonstrated in Refs. 8 and 9. This significantly extends the capabilities of the holography of volume ensembles of particles. Finally, the pulsed holography allows the recording of moving particles to be performed.

All the capabilities of the holographic methods, mentioned above, are, in principle, unattainable for other methods. Just these capabilities have caused the interest in the use of pulse holography to study moving particles in a liquid, especially, plankton in its natural habitat. The information about the shape and size of plankton particles along with the possibility of resolving fine details provide for their identification. The data on the spatial resolution, number density, and motion of particles allows the investigation of the species diversity and state of plankton. This, in its turn, permits the assessment of the ecological state of the water ecosystem, as well as the prediction of the growth of fish population based on the information about plankton as a very important part of the food chain.

Two submersible holographic cameras, which were used for *in situ* studies of plankton, are known.[10–13] The HOLOCAM camera[12,13] allows one to simultaneously record the axial and off-axial holograms of the same volume of a medium and provides for large viewing angle of the holographic image of every particle. In the off-axial scheme, the volume is irradiated in the reflective mode. Therefore, the source is a pulsed laser with the high output energy (about 1 J) and, consequently, the camera is characterized by large mass (more than 2 t) and overall dimensions. The information obtained in this case is excessive for most of the above problems. As a rule, to measure the size, number density, and positions of particles and to identify them, it is sufficient to measure the particle cross sections and avoid examination of holographic images in a wide range of observation angles.

This is characteristic, for example, of plankton of Lake Baikal with narrow species diversity. At the same time, in Lake Baikal there are no vessels, from which it is possible to work with heavy submersible instrumentation, what restricts possible investigations to only the work from ice. Therefore, it is urgent both to design a submersible holographic camera with smaller mass and dimensions and to develop the techniques for identification of plankton particles by their cross sections.

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In order to decrease the camera mass and dimensions, as well as the needed energy of the pulsed laser, we have chosen the off-axial scheme with image transfer and the transmission realization of the object beam.[7] In designing the camera, we based on the design solutions developed in NIPI Okeangeofizika, which allow the immersion depth to be increased considerably. The features of holographic recording with the submersible camera, the results of numerical simulation and calculation of the optical scheme of image transfer, the results of laboratory modeling of the optical scheme of holographic recording, as well as examples of holographic images are presented in Refs. 7, 14–16.

Simulator of a submersible camera

Before describing the simulator, let us remind briefly the functional diagram of the submersible holographic camera.[7] A part of the optical system is in a fast cylindrical housing, while another one is in two external cylindrical rods. The fast housing and the rods are arranged perpendicularly. The camera includes two additional fast cylindrical housings (for the laser and interface), located on two sides of the main case along it and intended for housing the laser, power supply, and central controller. The volume under study is located between the two external rods. It is a cylinder of 50-mm diameter and 500 mm length. The studied volume is spaced by about 700 mm from the main fast housing to minimize the turbulent distortions.

To develop the technique of underwater holographic recording and the technologies of assembling the submersible holographic camera, alignment of the optical system, recording the hologram, loading photoplates, shooting, maintaining the laser system, etc., we have made the construction, called a simulator of the submersible holographic camera (Fig. 1).

In its idea, the simulator is a full-size autonomous laboratory model of the submersible holographic camera. The laser box, the main solid housing, and both rods (transmitting and receiving) are replaced by tubes of the corresponding diameter, installed on the common platform. The cell with water (length of 500 mm), placed between the rods and illuminated by the object beam, imitates the volume under study. There is no interface box in the simulator, since the power supply and the central controller, installed in it, do no need mechanical alignment and adjustment.

All units, which are mounted inside the simulator housings, will be installed, after testing and revision, in the working submersible version of the holographic camera.

Thus, this simulator includes the complete set of optical components of the submersible holographic camera, we have designed earlier.[7]

A beam-turning mirror *1* directs the laser beam to a beam-splitter *2*. The beam, passed through the beam splitter, is expanded by a Galilean telescopic system *3* to the diameter of 50 mm. Then it illuminates, in the transmission mode, the cell with water *5*, which houses the studied objects, and comes to an image transfer system *6*. The system *6* is a Keplerian telescopic system. It transfers the image of a studied object into the area in front of a photoplate *8*. Its use in the submersible holographic camera increases the scene depth. The radiation, reflected by the beam splitter *2*, after the following reflection from the system of beam-turning mirrors comes to a collimator *4* (identical to a collimator *3*), where it forms the reference beam. After the exit from the collimator, the beam 50 mm in diameter, having passed through a system of mirrors *7*, illuminates a photoplate *8* at an angle of 30°. The function of the system of mirrors, as well as the system of mirrors in the scheme of the submersible camera, is to direct the reference beam at a given angle to the photoplate, providing for the needed space for the photoplate storage and feed mechanism. Optical elements of the reference beam are installed on a sliding platform, which is shown in Fig. 1*e*. The design solutions of the plate, holders, and optical elements used provide for minimum misalignment of the optical layout of the simulator, when the plate is taken off and then set back into its working position.

The simulator employs a Nd:YAG laser with a passive switch, made of a LiF crystal with F^{2-} color centers, with the following amplification and frequency conversion to the second harmonic (by use of a KTP crystal). The laser uses a diaphragm to separate a single transverse mode and a resonance reflector as an output mirror, which provides for the needed coherence length. The output energy obtained in the experiments is low and amounts to about 30 mJ. However, the optical elements of the holographic channel calculated and fabricated for the minimum loss, as well as the use of the transmission mode of irradiation by the object beam have allowed us to restrict the experiments to this energy.

The experiments carried out with the use of the simulator have shown that this energy is sufficient for the hologram recording by use of the camera layout shown in Fig. 1. (Remind that in Refs. 5, 6, 12, and 13 the laser output energy was an order of magnitude higher.) The features of our laser are small size and simple design and, consequently, stability to mechanical disturbances. At the same time, whenever necessary, it can be equipped with a telescope and a second amplifier. The appearance of the laser, installed in the simulator of the laser box, is shown in Fig. 1*b*.

Experiment

In the experimental investigations, we used the following objects: a wire of 450 μm in diameter, live moving species of *Tubifex* (diameter of about 800 μm),

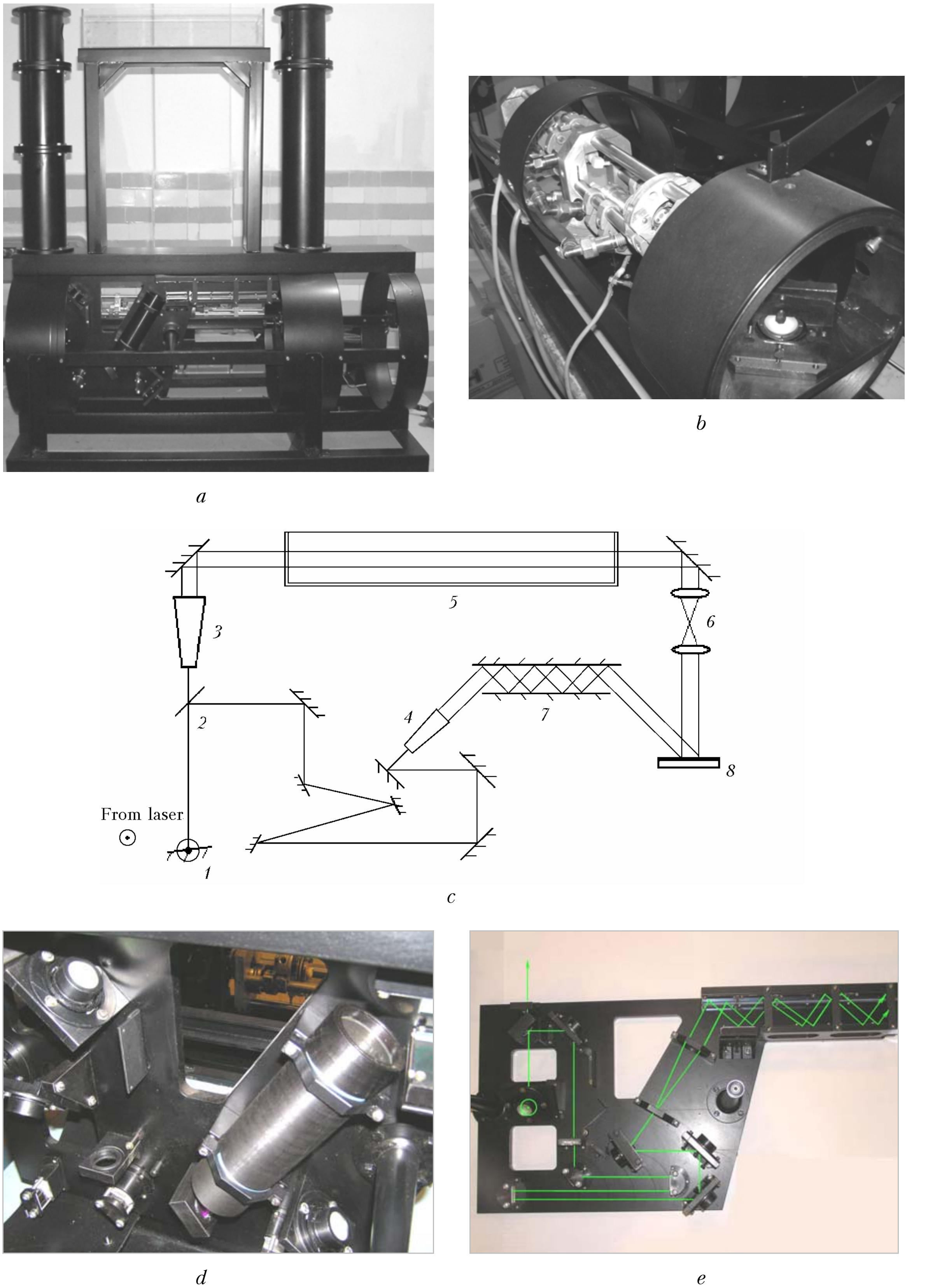


Fig. 1. General view of the simulator (*a*, *b*) and its optical arrangement (*c*); module for formation of the object beam: (*d*) as a part of the simulator; (*e*) separately from the simulator; the light line shows the beam path; (*c*) beam-turning mirror *1*, beam splitter *2*, collimators (Galilean telescopic system) *3* and *4*, glass cell modeling the studied volume *5*, optical system of image transfer (Keplerian telescopic system) *6*, mirror system *7*; photoplate *8*.

and plankton particles from Lake Baikal. The objects were placed in the cell with water (5, Fig. 1*c*) at different distances (50, 250, and 450 mm) from the exit window of the cell.

After the exposure and photochemical processing, the recorded holograms were reconstructed on a separate laboratory bench, whose optical arrangement is shown in Fig. 2.

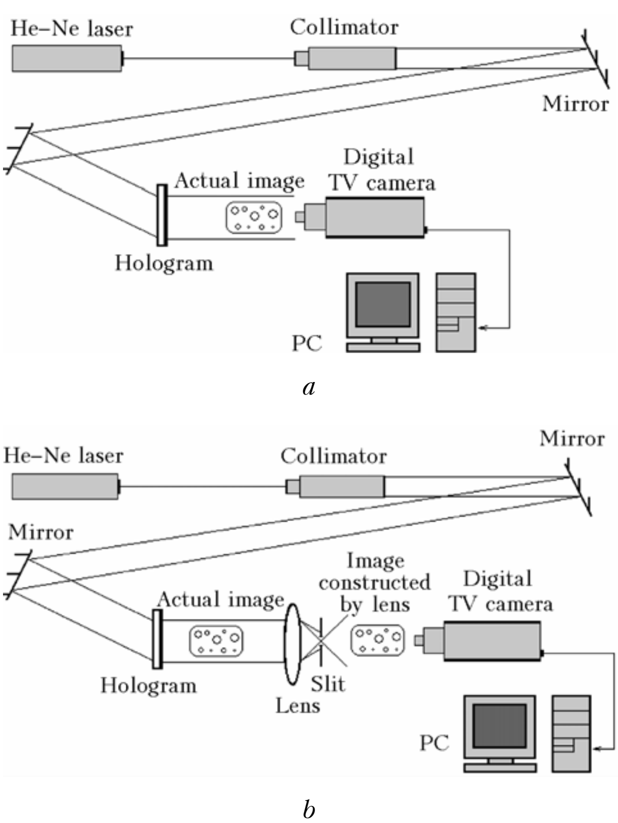


Fig. 2. Scheme of reconstruction of holographic images (*a*); scheme with filtering of spatial frequencies (*b*).

A cw He–Ne laser was used as a source of radiation. At the exit from the collimator, the beam was expanded to the diameter of 50 mm. Two beam-turning mirrors provide for the exact adjustment of the incidence angle of the reconstructing beam onto a hologram based on the best quality of the reconstructed image. The reconstructed actual holographic images of the objects studied could be observed though a microscope and/or saved in the computer in the digital format (BMP files) using a system for entering the holographic images. This system included a digital CCD camera with a specially developed attachment. The attachment allowed us to install microobjectives with different magnification. In addition, the system was equipped with a video card with S-Video interface to catch the video signal.

Figure 3 depicts the photographs of different planes of the reconstructed volume holographic images of *Tubifex* species moving in water (Fig. 3*a*) and plankton particles (Fig. 3*b*) with different magnification.

Based on the known sizes of these biological objects, we can say that the resolution achieved was 200 μm, which agrees with the calculated results.[7] Figure 4 shows the photographs of the reconstructed holographic images of model 100-μm sized objects, being in water at a distance of 50 (*a*), 250 (*b*), and 400 mm (*c*) from the exit window of the cell.

As already mentioned, the contrast is an important parameter of reconstructed holographic images. To increase the contrast of the reconstructed images by the instrumental method, the filtering of spatial frequencies was applied at the stage of reconstruction. For realization of this method, the optical layout (see Fig. 2*a*) was supplemented with a lens, in focal plane of which we placed a slit (see Fig. 2*b*).

The use of the system for entering the holographic images into the computer allows their further processing by numerical methods. Figure 5 shows the results of processing of the reconstructed images by a filter of spatial frequencies both instrumentally and numerically, with the use of numerical filtering.

The numerical processing is obviously preferred as compared to the instrumental one, since it provides wider possibilities for the selection of the filtering parameters and does not require any additional optical components. It should be noted that the numerical processing has its own limitations, connected with the computer resources, the limited data array of the CCD camera, etc.

To identify living objects, recorded with the aid of holographic methods, it is necessary to determine their shape, and to do this one should be capable of automatically (semi-automatically) detecting image boundaries. Once the contrast of the reconstructed images is increased, the algorithm, detecting the boundaries of the studied objects, can be applied. The examples of operation of this algorithm are shown in Fig. 5.

Conclusions

The recording of holograms by the simulator designed has confirmed the tentative calculated estimates of the resolution (200 μm) and the scene depth (500 mm) of the submersible holographic camera for plankton investigations. It has been shown experimentally that the needed energy of the laser source can be restricted to 30 mJ, which is an order of magnitude lower that the source energy in analogous cameras. The experiments carried out have revealed the need in the development of the optimal procedure entering the volume holographic images into a computer and their processing.

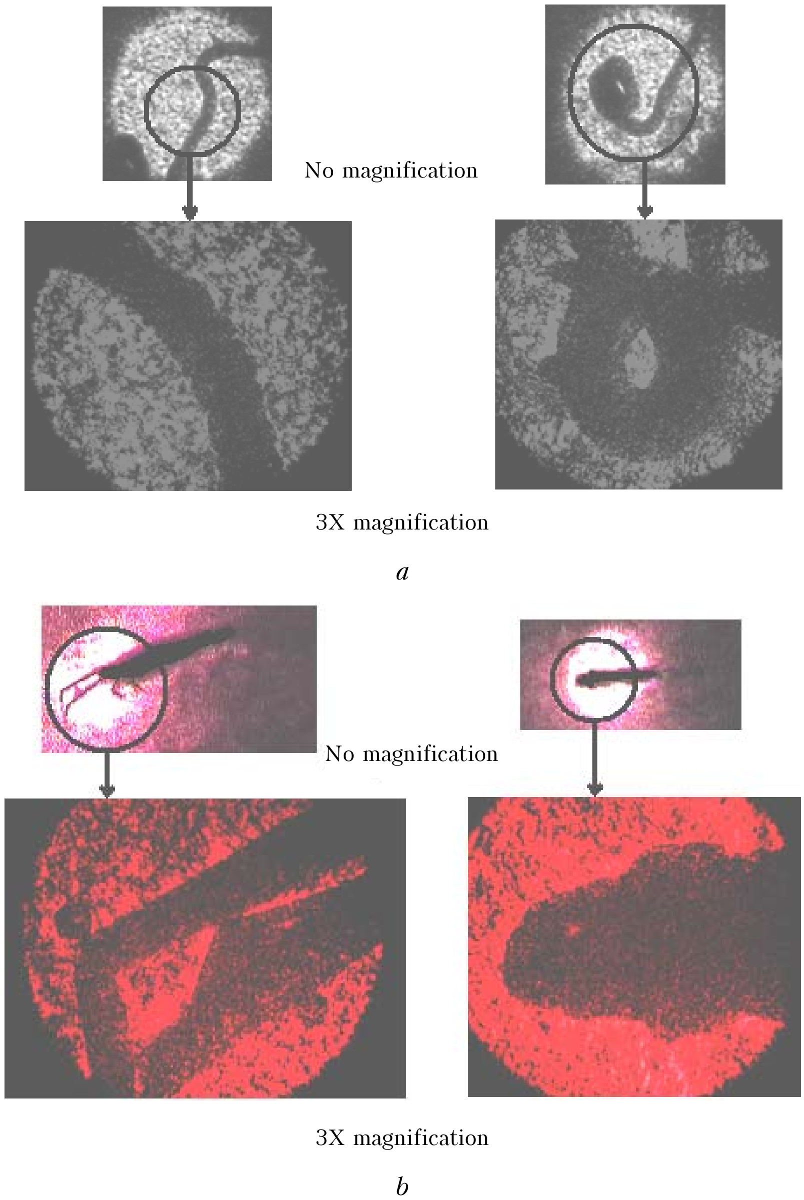
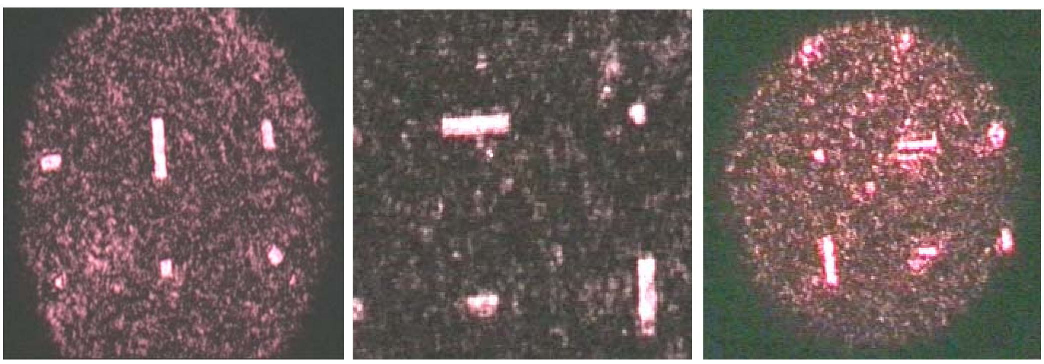


Fig. 3. Reconstructed holographic images: (*a*) actual holographic image of a *Tubifex* individual (diameter ~ 800 μm), moving in water; (*b*) reconstructed holographic images of plankton particles of Lake Baikal.



a *b* *c*

Fig. 4. Reconstructed holographic images of model objects.

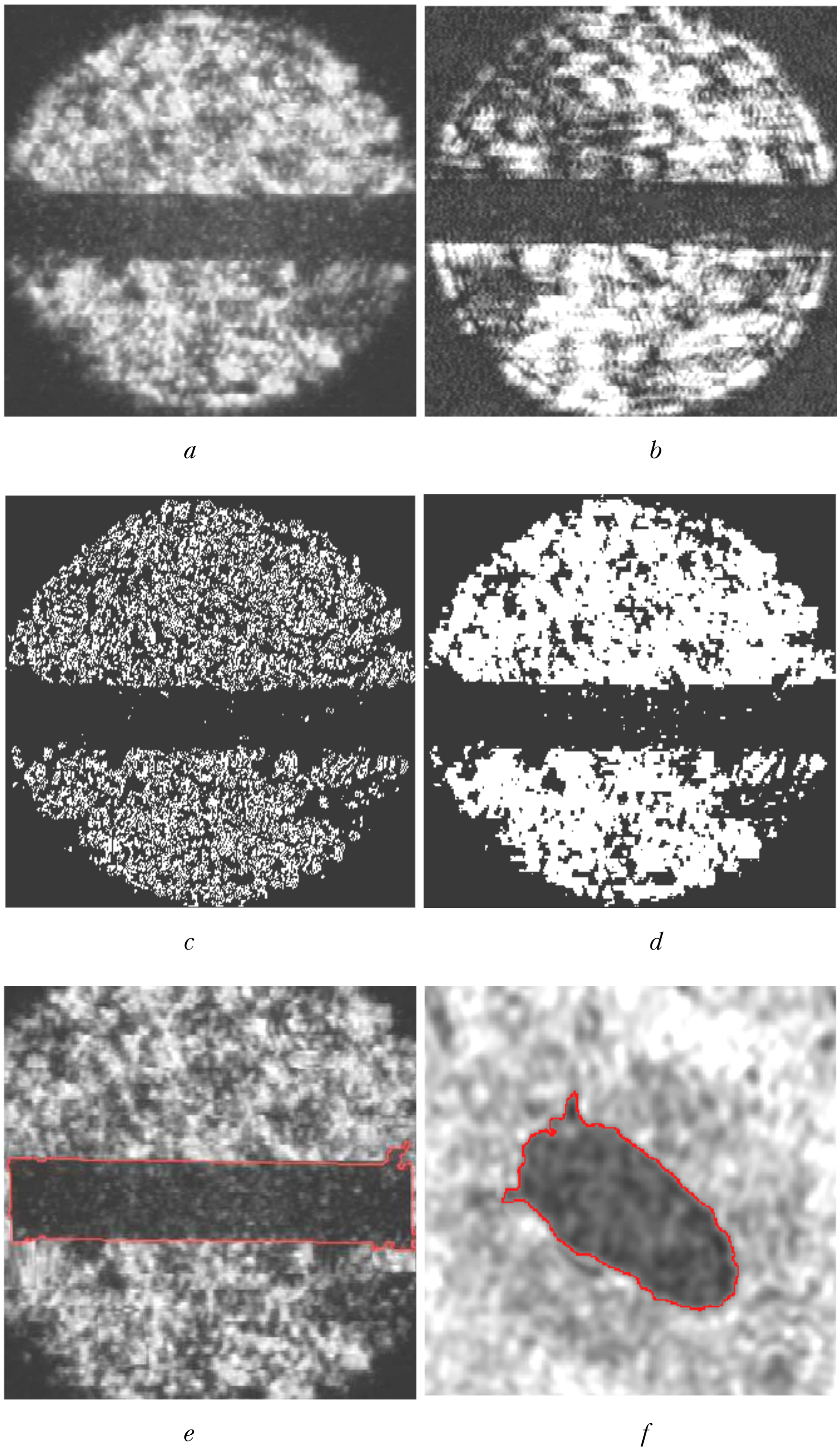


Fig. 5. Examples of the instrumental and numerical processing of holographic images, including the detection of boundaries: (*a*) initial image; (*b*) result of the instrumental processing using the optical set up shown in Fig. 2*b*; (*c*) numerical processing by the gradient filter; (*d*) numerical processing by the Sobel's filter; (*e*, *f*) results of operation of the algorithm, detecting the boundaries.

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