ЦИФРОВ ХОЛОГРАФСКИ МИКРОСКОП С ГОЛЯМО УВЕЛИЧЕНИЕ
HIGH MAGNIFICATION DIGITAL HOLOGRAPHIC MICROSCOPE

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Abstract

Rapid progress in electronic detection and control, digital imaging, image processing, and numerical computation has been crucial in the advance of modern microscopy. Digital holographic microscopy is a new advanced imaging technology which yields a 3D volume image from a single image capture.

In conventional holography, invented by Gabor, the holograms are photographically recorded and optically reconstructed. Both the amplitude and phase information of the light wave are recordedin a hologram.

A high magnification digital in-line holographic microscope was developed at the Agricultural University of Plovdiv. The microscope was calibrated using an USAF target. The digital microscope was successfully used to visualize nanoparticles with dimension of 2 µm.The numerical reconstruction of the digital holograms was done using the appropriate software.

Key words: digital holographic microscopy, nanoparticles, calibration.

INTRODUCTION

Gabor (Gabor, 1948) realized that the interference picture of a wave going through an object or reflected by it (object wave) with the initial undisturbed wave (reference wave) holds the whole information of the sample in it.

In his seminal paper he proposed an in-line assembly where the object and reference waves propagate in the same direction. The resulting superposition of both waves is recorded on a photographic plate. The emerging hologram is reconstructed by a new reference beam of same wavelength and the real image is obtained behind the plate.

The modern variant of Gabor holography use a CCD camera (or CMOS) to record the hologram and computer based mathematical methods to recover and magnify the image of the object.

Holography and speckle interferometric methods which are inherently linked to each other has become today a well-established tool for industrial nondestructive analysis and control of the quality. Using laser light as a powerful coherent source huge advance was made for enhancing the quality of the holograms, which boosted their implementation in practice (Maiman, 1960; Leith and Upatnieks, 1962; 1963; 1964; Powell and Stetson, 1965; Ostrovsky et al., 1980; Ostrovsky et al.,1991; Beeck and Hentschel, 2000; Shchepinov and Pisarev, 1996; Kreis, 1996; von Bally, 1979). Holographic methods have been applied in many engineering areas: i.e. in detection of displacements when applying static stress or shock waves, temperature changes or vibration monitoring (Ostrovsky, 1980; Ostrovsky, 1991; Beeck 2000; Shchepinov 1996; Kreis, 1996). Routinely the basic holographic techniques have been applied in the fields of biomedicine and life sciences (von Bally, 1979; von Bally and Greguss, 1982; von Bally, 2002) as well as in new research areas such as biophotonics (von Bally et al., 2008). A great advantage of the holographic and speckle interferometric metrology for biomedical applications is that they open up new potential for visualization as these techniques can be applied nondestructively, marker free, full field and online.

MATERIALS AND METHODS

Optical setup of the holographic microscope

Digital in-line holographic microscope (DIHM) was developed at the Agricultural University of Plovdiv (Peruhov and Mihaylova, 2012).

The light source is a diode laser (*Lasiris*) with wavelength of 673.2 nm and output of 7 mW. The laser radiation is focused onto a pinhole after which the intensity is controlled by a polarizer (Fig. 1). After the pinhole the spherical wave passes through the object: the diffracted by the object and the non-diffracted wave interfere and are recorded as a hologram on a CCD sensor. The intensity and the phase are reconstructed numerically (Mozhdeh Seifi et al., 2012).

Using the setup discussed above holograms of microparticles are obtained and subsequently their amplitude and phase are reconstructed using the software package (Mozhdeh Seifi et al., 2012).

It was found experimentally that the optimum distance between the laser source and the objects is 3 cm to record hologram of the micro-particles. The distance from the object to the CCD camera in our experiments is 15 cm. These distances determine the magnification of the microscope. The reference wave influence has been eliminated by subtraction.

USAF Test Target 1951 was used to calibrate the holographic microscope. Table 1 presents the characteristics of this USAF Test Target 1951. Element 2 of group 2 was used for the calibration because the elements of this group fill in the full field of view by the magnification received.

RESULTS AND DISCUSSION

Two groups of experiments were conducted. The first one was the calibration with the use of the USAF Target. Digital holograms were taken of element 2 group 2. A hologram is presented on Fig. 2 and the amplitude reconstruction and phase reconstruction are shown respectively on Fig. 3 and Fig. 4.

The second group of experiments were performed with micro-particles – nonporous silica (SiO_2) particles having average diameter 2.06 ± 0.13 µm. The micro-particles were produced by *Microparticles GmbH*, Berlin, Germany. The aim of these experiments was to visualize objects with sizes in the region 1–5 micrometers by means of digital holography. Figures 5–7 represent the holograms of the micro-particles. Three figures are presented to demonbstrate repeatability of the experiment. Figures 8–10 show the images of digital intensity reconstruction of parts of the holograms. It was chosen to reconstruct square areas around single micro-particles.

CONCLUSIONS

1. A high magnification digital in-line holographic microscope was developed at the Agricultural University of Plovdiv. The microscope was calibrated using an USAF target. It was demonstrated that digital in-line holographic microscopy can be routinely used to visualize microparticles with sizes in the region 1–5 micrometers**.**

2. The new microscope can be successfully used in life sciences to visualize objects having dimensions in the region 1–10 micrometers**.** The reported development will be very beneficial for investigations of biological samples in the agricultural sciences, because the digital microscopy does not require any staining or other preliminary preparation of the samples.

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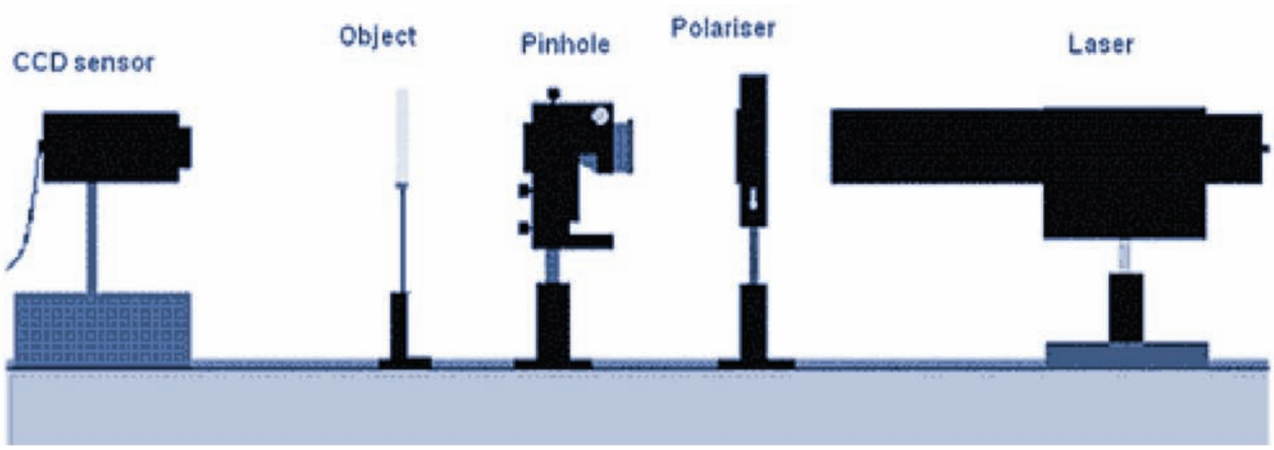

Fig. 1. *Optical set-up of the digital in-line holographic microscope*

Table 1. Characteristics of the USAF Test Target 1951

Element	-2	-1	0	1	2	3
1	2000	1000	500	250	125	62.5
2	1785.71	891.27	446.43	223.21	111.36	55.68
3	1587.30	793.65	396.83	198.41	99.21	49.50
4	1416.43	707.21	354.61	176.68	88.34	44.25
5	1259.45	630.52	314.47	157.73	78.74	39.37
6	1123.60	561.17	280.90	140.45	70.13	34.97

Calibration of the holographic microscope

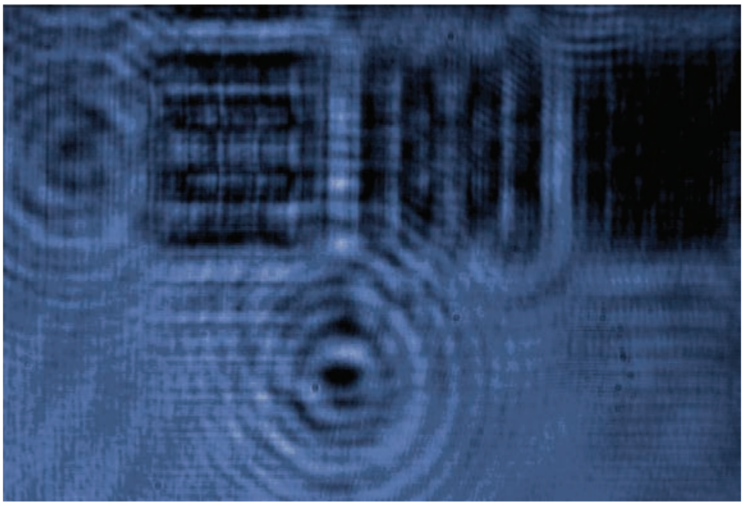

Fig. 2. *Hologram of USAF Target 1951 (group 2 element 2)*
The field of view is 500 x 340 micrometers

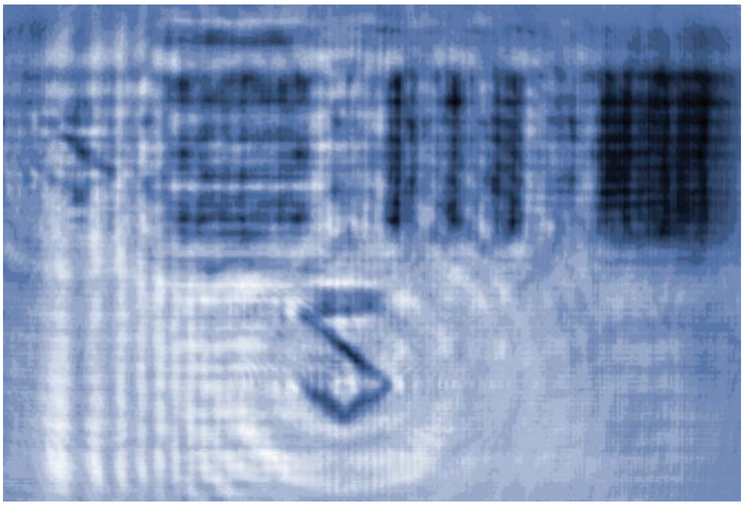

Fig. 3. *Amplitude Reconstruction of the hologram of USAF Target 1951 presented on Fig. 2*

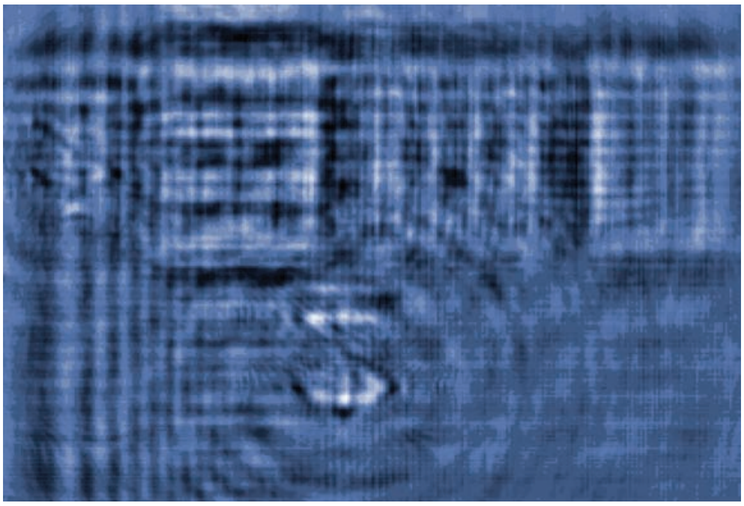

Fig. 4. *Phase reconstruction of the hologram of USAF Target 1951 presented on Fig. 2*

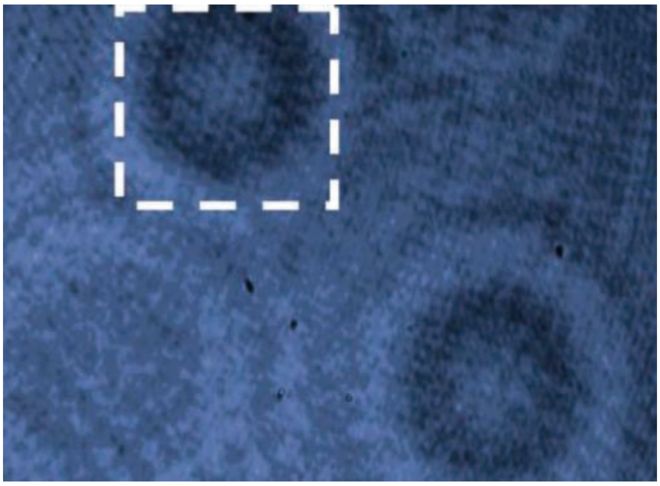

Fig. 5. *Hologram of two nonporous silica (SiO_2) micro-particles known to have an average diameter of 2.06 ± 0.13 µm. The dashed square area of the hologram was chosen for reconstruction*

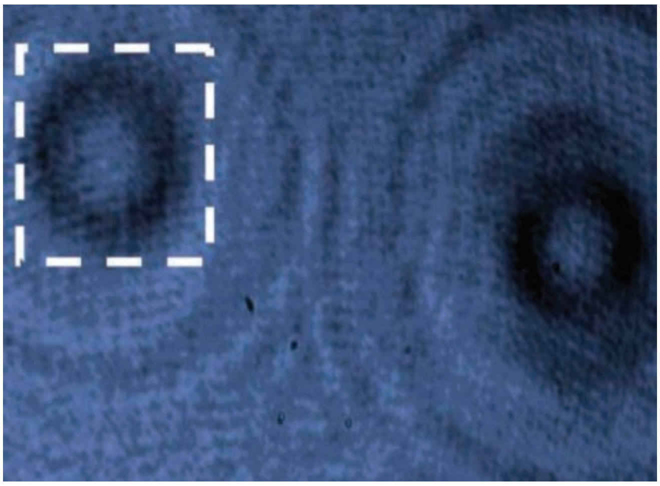

Fig. 6. *Hologram of two nonporous silica (SiO_2) micro-particles known to have an average diameter of 2.06 ± 0.13 µm. The dashed square area of the hologram was chosen for reconstruction*

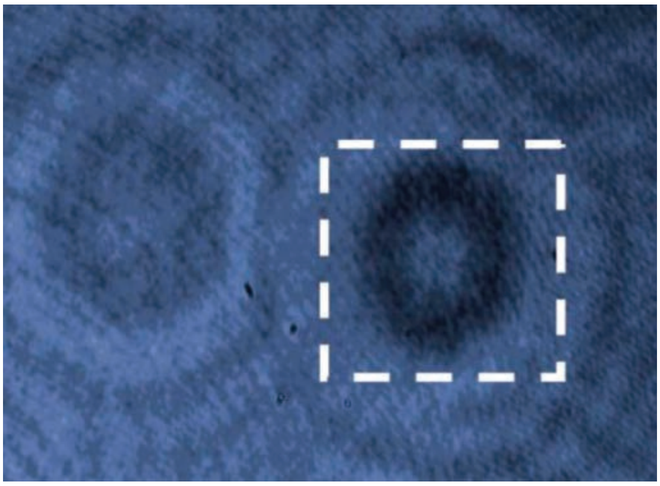

Fig. 7. *Hologram of two nonporous silica (SiO_2) micro-particles known to have an average diameter of 2.06 ± 0.13 μm. The dashed square area of the hologram was chosen for reconstruction*

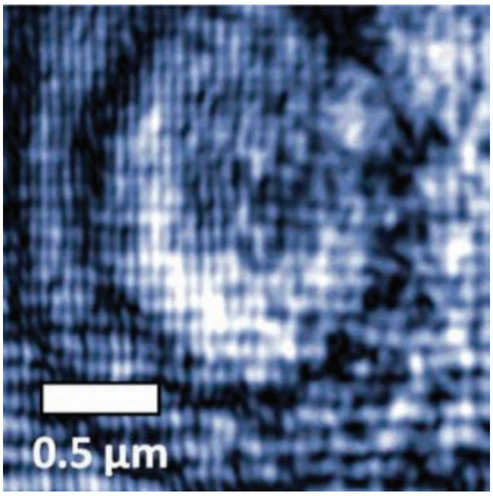

Fig. 8. *Digital intensity reconstruction of the dashed square area of the hologram shown in Fig. 5*

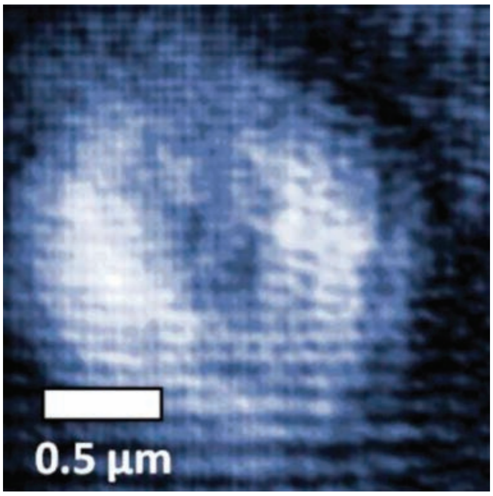

Fig. 9. *Digital intensity reconstruction of the dashed square area of the hologram shown in Fig. 6*

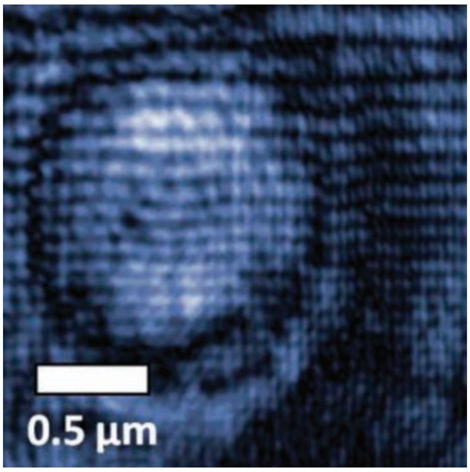

Fig. 10. *Digital intensity reconstruction of the dashed square area of the hologram shown in Fig. 7*

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