ISSN 8756-6990, Optoelectronics, Instrumentation and Data Processing, 2018, Vol. 54, No. 3, pp. 301–306. © Allerton Press, Inc., 2018.
Original Russian Text © V.I. Guzhov, S.P. Il'inykh, I.O. Marchenko, 2018, published in Avtometriya, 2018, Vol. 54, No. 3, pp. 104–110.

OPTICAL INFORMATION TECHNOLOGIES

Method of Increasing the Spatial Resolution in Digital Holographic Microscopy

V. I. Guzhov*, S. P. Il'inykh, and I. O. Marchenko

Novosibirsk State Technical University,
pr. Karla Marksa 20, Novosibirsk, 630073 Russia
**E-mail: vigguzhov@gmail.com*

Received December 26, 2017

Abstract—A new method of increasing the spatial resolution in digital holographic microscopy is considered. The method is based on supplementing the initial hologram with results measured in the case of photodetector shifting in space by a value smaller than the resolution used. In contrast to other known approaches, this method does not require a system of equations to be solved.

Keywords: digital holography, holographic microscopy, interferometry, spatial shift, super-resolution, synthesized aperture.

DOI: 10.3103/S8756699018030135

INTRODUCTION

The holographic method appeared due to improvement of electron microscopy. The idea was to write a hologram of a microscopic object by using an available coherent source of electrons [1]. If this hologram was illuminated by coherent light waves in the course of reconstruction, obtaining a significantly increased three-dimensional image of this microscopic object was theoretically predicted. However, this idea was not realized because of difficulties in working with electron beams [2].

Modern holographic microscopes are based on schemes of image zooming with the use of micro-objectives, as in usual optical microscopes [3, 4]. Figure 1 shows the optical scheme of a digital holographic microscope [5]. The object image is reconstructed in the Fresnel or Fraunhofer domain, depending on the distance d.

The main advantage of such systems is enhancement of the range and accuracy of profile (z coordinate) measurement, whereas the spatial resolution (in the x and y directions) remains the same as that in optical microscopy. This resolution is determined by the Rayleigh criterion [6]

$$R = 0.61(\lambda/NA^{\mathrm{obj}}), \tag{1}$$

where NA^{obj} is the numerical aperture, which depends on the micro-objective structure. The maximum theoretical value of the numerical aperture in air cannot exceed unity. Higher values are reached by placing an immersion medium between the frontal lens and the object. In this case, the value of NA^{obj} can be slightly greater than unity.

In practice, for the wavelength of the order of 500 nm and numerical aperture of 1.4, the resolution along the x and y axes does not exceed 200 nm. At the same time, the spatial resolution of electron microscopes can be smaller than 1 nm. Therefore, the attention of many researchers is riveted to various issues of synthesizing the image from a set of low-resolution patterns obtained by means of shifting the object image by a subpixel value [7–10]. Such an approach is called the aperture synthesis in radio engineering and "super-resolution" methods in optics. In the present paper, a new method of increasing the resolution in digital holography is proposed, which does not require a system of equations to be solved.

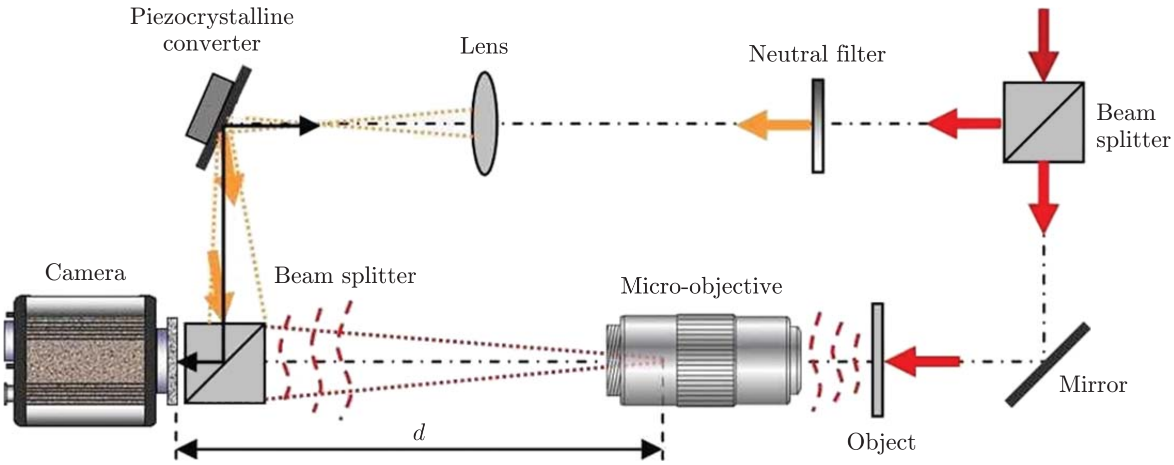

Fig. 1. Scheme of the digital holographic microscope.

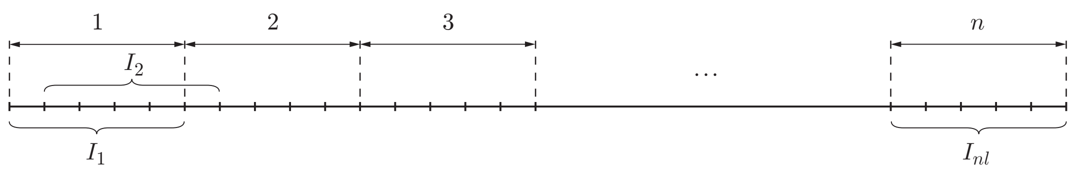

Fig. 2. Scheme of recording of the low-resolution signal in the case of a subpixel shift in one line.

FORMULATION OF THE PROBLEM

The general formulation of the problem of forming a super-resolution pattern was described in [11]. Figure 2 shows the scheme used for recording a one-dimensional signal in the case of its scanning by a low-resolution aperture. Here n is the number of elements of the low-resolution pattern, l is the number of high-resolution elements captured by the integrated aperture I_i, $i = 1, \ldots, n$, and nl is the number of elements in the high-resolution pattern.

The measurements yield a set of values with low resolution I_i, which are shifted with respect to each other by a certain value smaller than the size of the integrated aperture. The main task is to determine high-resolution elements x_i from the system of equations

$$\begin{aligned} &x_1 + x_2 + \ldots + x_l = I_1; \\ &x_2 + x_3 + \ldots + x_{l+1} = I_2; \\ &\ldots \\ &x_{(n-1)l+1} + x_{(n-1)l+2} + \ldots + x_{nl} = I_{nl}. \end{aligned} \tag{2}$$

As the number of the shifting events increases, the size of the system of linear equations becomes very large. The main investigations in this field are aimed at searching for methods that accelerate and stabilize the solution of system (2) and for methods that increase the computation speed.

ALGORITHM AND ITS IMPLEMENTATION

An optical system of digitizing holograms recorded on usual photoplates [12, 13] by using a modified Metam R-1 optical microscope produced by the LOMO company was developed for experimental modeling. Digitization was performed with a hologram obtained at an angle of 5° between the interfering reference and object beams. If a hologram region with a size of 3×3 mm is scanned with the number of elements 2048×2048 and resolution of 1.5 μm, then the chosen resolution is sufficient for reconstruction of the real and imaginary images (Fig. 3). As the hologram was recorded in the Fraunhofer domain, the image was reconstructed with the use of the Fourier transform [14, 15].

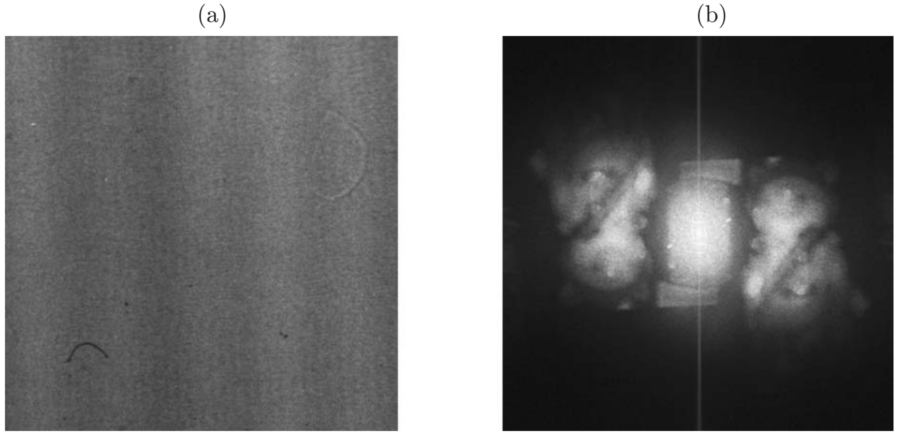

Fig. 3. Hologram reconstruction: (a) digitized hologram with averaging over the vicinity of 3×3 points (2048×2048); (b) real and imaginary images reconstructed from this hologram.

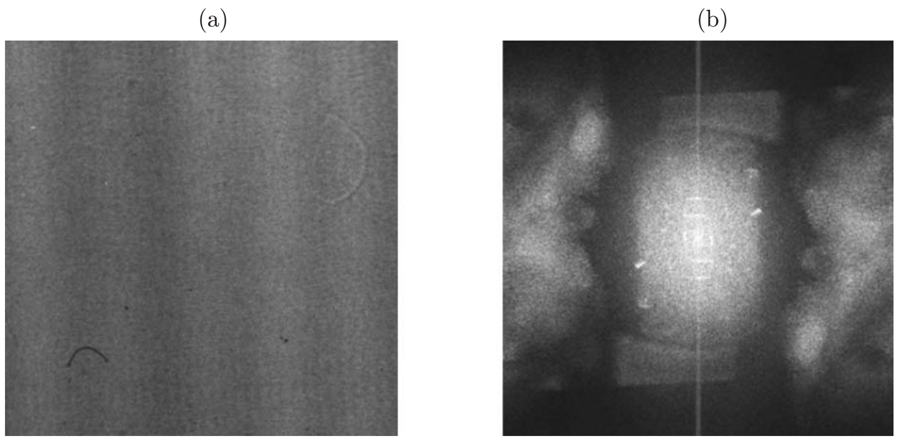

Fig. 4. Hologram reconstruction in the case of low resolution: (a) hologram with the resolution of 1024×1024; (b) reconstructed image.

However, if the resolution is reduced to 3 μm, then complete reconstruction of the real and imaginary images is impossible for the chosen angle between the interfering bands. The image portions corresponding to the high frequencies in the spectrum disappear (Fig. 4).

If the hologram (or detector) can be moved in the object area of the microscope by a value smaller than the chosen resolution, then the images corresponding to the high resolution during digitization can be reconstructed from a series of holograms digitized with insufficient resolution. Let the results of digitization of four holograms with the resolution of 1024×1024 be available (Fig. 5): without shifting $A(x, y)$; with a shift along the X axis by a value equal to one half of the resolution $AX(x, y)$; with a shift along the Y axis $AY(x, y)$; finally, with a shift along both the X and Y axes $AXY(x, y)$. In this case, we can form a matrix of high-resolution elements from the system of equations (2).

The values of $A(x, y)$, $AX(x, y)$, $AY(x, y)$, and $AXY(x, y)$ can be obtained by applying three shifts. First, the frame A with no shift is fixed; then it is shifted by one half of the resolution element to the right and the frame AX is obtained; then the resultant frame is shifted downward for obtaining the frame AXY; finally, the frame is shifted to the left and the frame AY is obtained. If the system resolution is 3 μm, then the shifting step should be 1.5 μm.

Let us form a matrix from the results of low-resolution measurements (see the table). Then the Fourier transform is applied for obtaining the real and imaginary images.

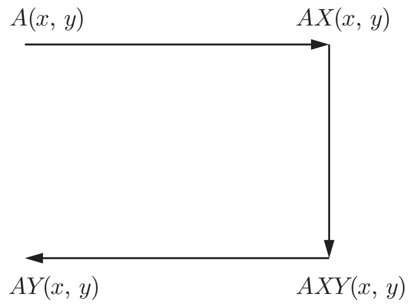

Fig. 5. Sequence of spatial shifts with resolution doubling.

Formation of a hologram from a set of low-resolution holograms

$A(x_0, y_0)$	$AX(x_0, y_0)$	$A(x_1, y_0)$	$AX(x_1, y_0)$	$A(x_2, y_0)$	$AX(x_2, y_0)$	...
$AY(x_0, y_0)$	$AXY(x_0, y_0)$	$AY(x_1, y_0)$	$AXY(x_1, y_0)$	$AY(x_2, y_0)$	$AXY(x_2, y_0)$	...
$A(x_0, y_1)$	$AX(x_0, y_1)$	$A(x_1, y_1)$	$AX(x_1, y_1)$	$A(x_2, y_1)$	$AX(x_2, y_1)$	...
$AY(x_0, y_1)$	$AXY(x_0, y_1)$	$AY(x_1, y_1)$	$AXY(x_1, y_1)$	$AY(x_2, y_1)$	$AXY(x_2, y_1)$	...
$A(x_0, y_2)$	$AX(x_0, y_2)$	$A(x_1, y_2)$	$AX(x_1, y_2)$	$A(x_2, y_2)$	$AX(x_2, y_2)$	...
$AY(x_0, y_2)$	$AXY(x_0, y_2)$	$AY(x_1, y_2)$	$AXY(x_1, y_2)$	$AY(x_2, y_2)$	$AXY(x_2, y_2)$	...
$A(x_0, y_3)$	$AX(x_0, y_3)$	$A(x_1, y_3)$	$AX(x_1, y_3)$	$A(x_2, y_3)$	$AX(x_2, y_3)$	...
$AY(x_0, y_3)$	$AXY(x_0, y_3)$	$AY(x_1, y_3)$	$AXY(x_1, y_3)$	$AY(x_2, y_3)$	$AXY(x_2, y_3)$	...
...	...	...	...	...	...	...

Figure 6 shows a hologram with a size of 2048×2048 pixels, which was formed from a set of four low-resolution holograms (1024×1024) and the real and imaginary images reconstructed from the initial hologram. It is seen that the resolution correspond to hologram digitization in the case of its doubling. Increasing the resolution by a factor of 3 requires eight shifts and nine low-resolution frames.

Figure 7 shows a hologram with a size of 2048×2048 pixels, which was generated from a set of nine low-resolution holograms (682×682) and the real and imaginary images reconstructed from this hologram. Such a procedure can be extended to an arbitrary number of steps. The size of the generated hologram will be n^2 times greater than the initial hologram, where n is the number determining the increase in the resolution. In this case, the resolution that can be reached is determined only by the magnitude of shifting.

A constraint of this method is a long time needed for computations. For example, if the field of vision of the microscope is digitized on a frame 1000×1000 pixels with a resolution of 1 μm, then one has to apply the Fourier transform to an array of $nl \times nl = 1000000 \times 1000000$ elements in order to reach a resolution of 1 nm. To reduce the computation time, it is recommended to perform such operations on graphics processing units.

Another hardware-associated constraint is the complexity of setting the exact value of the subpixel shift. Modern positioning devices use piezo-ceramics [16, 17], which induces significant errors because of the hysteresis effects. Figure 8 shows the result of reconstructing the hologram synthesized on the basis of the low-resolution holograms recorded with an error in shift setting, which was 1/4 instead of the expected value (by one half of a pixel). There are geometric distortions in the reconstructed images; nevertheless, the resolution still allows reconstruction of image portions corresponding to high harmonics. Thus, the algorithm is sufficiently stable to shift setting errors. However, the geometric distortions caused by incorrect setting of the shift should be taken into account in quantitative calculations.

CONCLUSIONS

A new method of increasing the spatial resolution in digital holographic microscopy is considered. In holographic microscopy, the images are reconstructed in the Fresnel or Fraunhofer domain; therefore, the Fourier transform should be applied to the hologram recorded in the object domain for the purpose of image reconstruction. In this case, it is not necessary to solve the system of equations. The method is based on

(a) (b) (c)

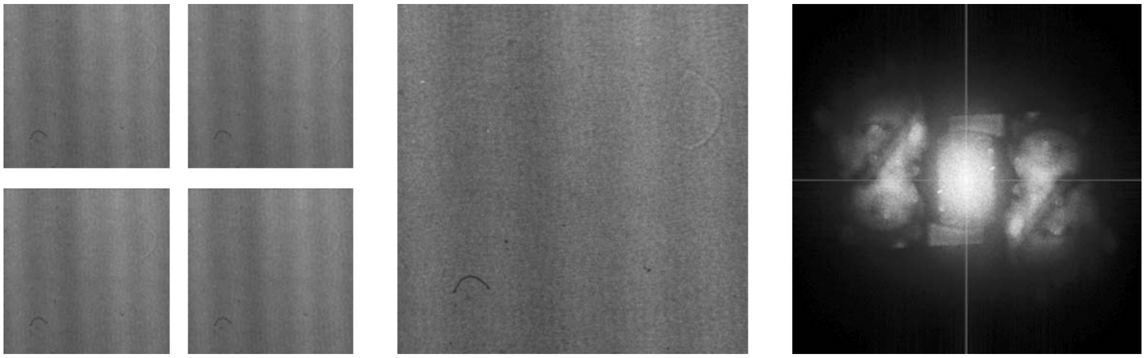

Fig. 6. Increase in the resolution in the case of subpixel scanning of the hologram: (a) four holograms (1024 × 1024) with a shift by one half of a pixel; (b) synthesized hologram 2048 × 2048 pixels; (c) image reconstructed from the synthesized hologram.

(a) (b) (c)

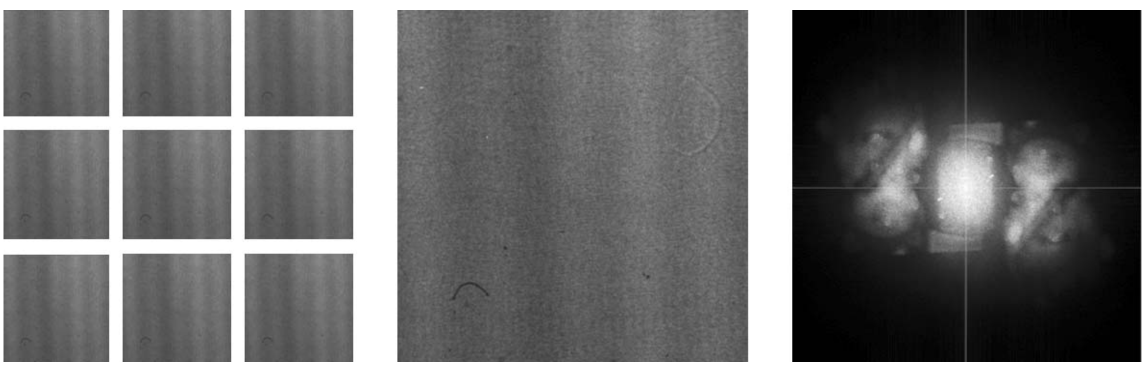

Fig. 7. Increase in the resolution by a factor of 3 in the case of subpixel scanning of the hologram: (a) nine holograms (682×682) with a shift by 1/3 of a pixel along the X and Y axes; (b) synthesized hologram 2048 × 2048 pixels; (c) image reconstructed from the synthesized hologram.

(a) (b)

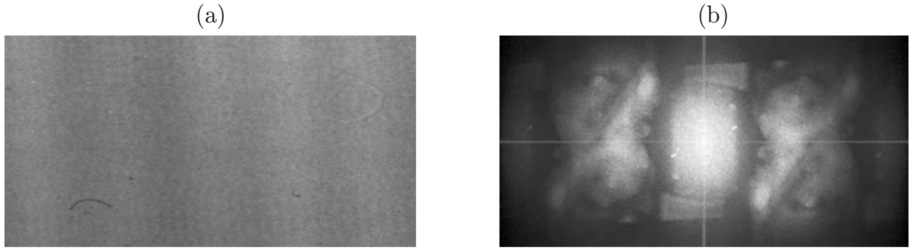

Fig. 8. Increase in the resolution along the X axis in the case of subpixel scanning of the hologram with incorrect setting of the spatial shift: (a) synthesized hologram 2048 × 1024 pixels; (b) image reconstructed from the synthesized hologram.

supplementing the initial hologram with results measured in the case of spatial shifting by a value smaller than the resolution used.

Modern positioning devices ensure shifting along the X and Y axes with a step down to 0.1 nm. In this case, it is possible to reach the spatial resolution of the optical system smaller than 1 nm.

The constraints of the new method are induced by the long computation time needed for calculating large arrays with the Fourier transform and geometric distortions of reconstructed images caused by errors in setting the shifts in space.

This work was supported by the Russian Foundation for Basic Research (Grant No. 18-08-00580)

REFERENCES

1. D. Gabor, "A New Microscopic Principle," Nature **161** (4098), 777–778 (1948).
2. V. I. Guzhov, *Mathematical Methods of Digital Holography: Tutorial* (Izd. NGTU, Novosibirsk, 2017) [in Russian].
3. M. Junwei, B. Yao, P. Gao, et al., "Parallel Phase-Shifting Interferometry Based on Michelson-like Architecture," Appl. Opt. **49** (34), 6612–6616 (2013).
4. L. Ma, Y. Li, H. Wang, and H. Jin, "Fast Algorithm for Reliability-Guided Phase Unwrapping in Digital Holographic Microscopy," Appl. Opt. **51** (36), 8800–8807 (2012).
5. V. Micó, C. Ferreira, Z. Zalevsky, and J. Garcia, "Basic Principles and Applications of Digital Holographic Microscopy," in *Microscopy: Science, Technology, Applications and Education*, Ed. by A. Méndez-Vilas and J. Diaz, Formatex Microscopy Ser. **2** (4), 1411–1418 (2010).
6. V. P. Koronkevich, *Image Formation in Optical Systems: Tutorial* (Izd. NGTU, Novosibirsk, 2005) [in Russian].
7. M. Paturzo, F. Merola, S. Grilli, et al., "Super-Resolution in Digital Holography by a Two-Dimensional Dynamic Phase Grating," Opt. Express. **16**, 17107–17118 (2008).
8. D. Claus, "High Resolution Digital Holographic Synthetic Aperture Applied to Deformation Measurement and Extended Depth of Field Method," Appl. Opt. **49** (16), 3187–3198 (2010).
9. A. E. Tippie, A. Kumar, and J. R. Fienup, "High-Resolution Synthetic-Aperture Digital Holography with Digital Phase and Pupil Correction," Opt. Express. **19** (3), 12027–12038 (2011).
10. S. V. Blazhevich and E. S. Selyutina, "Increase in the Digital Image Resolution with the Use of Subpixel Scanning," Nauch. Vedomosti BelGU. Ser. Mat. Fiz. **176** (5(34)), 186–190 (2014).
11. S. T. Vaskov, V. M. Efimov, and A. L. Reznik, "Fast Digital Image and Signal Reconstruction by the Minimum Energy Criterion," Avtometriya **39** (4), 13–20 (2003) [Optoelectron., Instrum. Data Process. **39** (4), 11–17 (2003)].
12. V. I. Guzhov, D. V. Il'timirov, D. S. Khaidukov, et al., "Modification of Optical Microscopes," Avtomatika Program. Inzheneriya **16** (2), 71–76 (2016).
13. V. I. Guzhov, E. N. Denezhkin, O. V. Chernov, and N. S. Zarubin, "Reconstruction of Images from Real Holograms Recorded on Photoplates," Avtomatika Program. Inzheneriya **19** (1), 76–80 (2017).
14. V. I. Guzhov, V. A. Emel'yanov, and D. S. Khaidukov, "Domain of Possible Application of the Discrete Fourier and Fresnel Transforms," Avtomatika Program. Inzheneriya **15** (1), 97–103 (2016).
15. V. I. Guzhov, S. P. Il'inykh, and S. V. Khaibullin, "Phase Information Recovery Based on the Methods of Phase Shifting Interferometry with Small Angles between Interfering Beams," Avtometriya **53** (3), 101–106 (2017) [Optoelectron., Instrum. Data Process. **53** (3), 288–293 (2017)].
16. *Scan Company, RatisXY(Z) Two-Coordinate Plane-Parallel Scanner*. http://www.nanoscantech.com/ru/products/stage/stage-76.html.
17. *Camera IQ. Vieworks VN — High-Resolution Video Cameras with the Pixel Shift Technology*. https://www.cameraiq.ru.