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ANALYSIS AND SYNTHESIS OF SIGNALS AND IMAGES

Resolution Enhancement in Optical Microscopy Using Subpixel Shifts

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Abstract—A new method of resolution enhancement in optical microscopy using the method of spatial subpixel shifts, i.e., shifts by some amount less than the resolution provided by the lens are considered. The resolution of optical microscopes is determined by the type of used lenses. Professional microscopes have a set of microlenses with different magnifications, which are mounted on turrets containing several lenses. Sometimes it makes more sense to use one lens instead of a set of microlenses if it is possible to provide subpixel shifts. An increase in spatial resolution is carried out using the subpixel shift technique. In this case, the spectrum of the feature is supplemented by a multiplier, the type of which depends on the type of lens aperture. To obtain high-resolution features, it is necessary to divide the Fourier spectrum of an image obtained from several subpixel-shifted images by a factor depending on the selected aperture. This factor is called the aperture function. The aperture function is determined by the type of used lens and can be its nameplate value. An experimental method for calibrating a lens (obtaining its aperture function) with low resolution ($8\times$) based on images obtained with higher resolution lenses ($40\times$) is shown. Once the aperture function is defined, one low-resolution lens can be used to produce images at any resolution less than the resolution of the selected high-resolution lens ($40\times$)

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INTRODUCTION

The general formulation of the problem of spatial resolution enhancement of an optical system (for example, a photomatrix) using a subpixel shift (by an amount smaller than the spatial resolution of the system) is described in [1]. Figure 1 shows a scheme for recording a one-dimensional signal when it is scanned using a certain aperture of finite size. If, as a result of measurements, it is possible to obtain sets of values $\{x\}$ averaged over the aperture $\{I\}$ with low resolution (low-resolution features) shifted relative to each other by a certain amount smaller than the size of the integrating aperture, then it is possible to reconstruct $\{x\}$ (high-resolution features) with a resolution equal to the shift value [2–5]. In our case, resolution refers to the total number of subpixels, into which the pixels of a photomatrix row can be divided.

It is necessary to reconstruct high-resolution features $\{x_i\}$, $i \in [1, \ldots, L \times N]$ from features $\{x\}$ averaged over a certain aperture $\{I_j\}$, $j \in [1, \ldots, L]$. Here, L is the number of high-resolution features $\{x\}$ in one aperture; and N is the number of apertures (cells) in a row of the photomatrix.

The authors of [6, 16] showed that the expression for a function that consists of elements averaged over a certain aperture $\{I_i\}$ in the Fourier domain is

$$F_{\tau,\Delta x}(\omega) = \left(\left[F(\omega) \otimes \operatorname{sinc}\left(\frac{\omega N}{2}\right) \right] \Im\left(\operatorname{rect}_\tau(x)\right) \right) \otimes \operatorname{comb}_{2\pi/\Delta x}(\omega), \quad (1)$$

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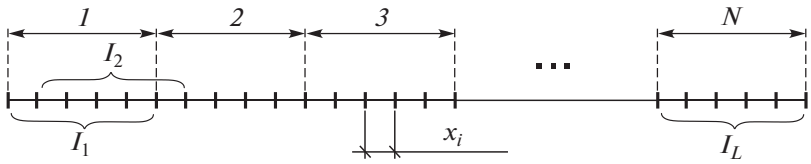

Fig. 1. Scheme for recording a signal averaged using apertures with a subpixel shift along one line [1].

where $F(\omega) \otimes \text{sinc}\,(\omega N/2) = \Im(f(x)) \otimes \text{sinc}\,(\omega N/2)$ is a discrete Fourier transform of a function consisting of $\{I_i\}$; $\Im\,(\text{rect}_\tau(x))$ is a Fourier transform of the aperture (aperture function); $\otimes$ is designation of the convolution operation; $\text{comb}_{2\pi/\Delta x}\,(\omega)$ is a Dirac comb, which is defined as a sequence of delta functions with a step of Δx; ω is a spatial frequency; and N is the number of delta functions in the Dirac comb.

It is clear from Eq. (1) that if the Fourier transform of an image from $\{I_i\}$ is divided by $\Im\,(\text{rect}_\tau(\omega))$, then one can obtain the Fourier transform of the original image consisting of $\{x_i\}$.

The purpose of this work is to experimentally obtain the aperture function for real optical microscopy systems and restore images with lenses that have insufficient resolution, with the same quality as when using lenses with higher resolution using the subpixel shift method.

EXPERIMENTAL

Development of a Modified Optical Microscope with an Ability to Introduce a Subpixel Shift

A disadvantage of optical microscopy systems is a fundamental limitation on the spatial resolution of the images. This resolution is determined by the Rayleigh criterion

$$R = 0.61\,\frac{\lambda}{NA^{obj}}, \tag{2}$$

where NA^{obj} is a numerical aperture, which depends on the design of the microlens; and λ is the wavelength of the light source. In practice, the spatial resolution of optical microscopes does not exceed 200 nm at a wavelength on the order of 500 nm [7, 8].

If it is possible to move an image in the microscope object area by an amount less than the resolution of the optical system, then the images with increased resolution can be reconstructed from a series of images digitized at low resolution. For this purpose, a METAM-R1 metallographic aggregate microscope was modified [9].

A Ratis XYZ_H plane-parallel scanning piezo stage [10] was used as the object stage. The use of this stage made it possible to provide a range of movement in the area *XY* 100 mm with a minimum movement step of 1 nm. For image sampling, a Canon EOS M50 serial digital mirrorless camera with an APS-C CMOS matrix at a resolution of 24.1 megapixels (6000 × 4000 pixels) was used. The matrix has a size of 22.3 × 14.9 mm, and the pixel size in the matrix is 3.7 μm. The camera is connected to the computer via a USB cable.

The appearance of the modified microscope is shown in Fig. 2.

The microlens always indicates the magnification factor and numerical aperture. The size of the numerical aperture depends on the design of the lens. Knowing the numerical aperture of the lenses, the resolution of the system can be determined (Table 1).

Experimental Determination of the Aperture Function

The aperture function was determined using test images, for which holograms obtained at different angles α between an object beam and a reference beam were used. The holograms were obtained according to the scheme proposed by Leith and Upatnieks [11–14].

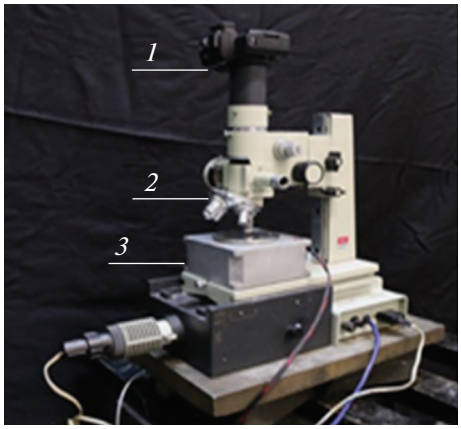

Fig. 2. Modified microscope. (*1*) A digital camera, (*2*) an optical microscope with a set of microlenses, and (*3*) an automated stage.

According to Kotelnikov's theorem, to correctly restore images from a hologram, it is necessary to take at least two samples per strip. The distance between adjacent stripes during the interference of two flat beams [15] is

$$\Delta x = \frac{\lambda}{2\sin(\alpha/2)}. \tag{3}$$

To demonstrate the capabilities, a hologram with an angle of 30° between the interfering beams was obtained (Table 2). An interferometer with a DSL6505-0921 laser module at a wavelength of 510 ± 5 nm was used. It follows from Table 2 that the chosen angle between the interfering beams satisfies the conditions of Kotelnikov's theorem.

Figure 3 shows a digitized hologram obtained at an angle of 30° between the interfering beams and images reconstructed from it obtained from the digitized hologram using a 40× lens. The hologram was recorded in the Fraunhofer region, so the image were restored using the Fourier transform.

It follows from Fig. 3 that the lens resolution of 40× ($R = 0.488$ μm) is sufficient to reconstruct a hologram with an angle of 30° between the interfering beams.

Now the aperture function $\Im(\mathrm{rect}_\tau(x))$ can be obtained as a result of element-wise division of the spectrum of the reconstructed image at a magnification of 8× by the spectrum of the image at a magnification of 40×. This approach can be justified by the fact that when recording a hologram of the same object using lenses with different magnifications, the spectra of the recorded holograms differ only in the type of spectrum of the lens aperture function (see expression (1)). Then, as a result of dividing

Table 1. System resolution when using LOMO lenses with different magnifications

Lens magnification	Lens resolution, μm	Size of the field of view on the camera matrix, mm	Aperture, a. u.
8×	1.83	2.42	0.17
20×	0.92	1.08	0.34
40×	0.49	0.54	0.64

Table 2. Required resolution for hologram input

Angle between interfering beams	Distance between stripes, μm	Required resolution, μm
30°	0.98	0.49

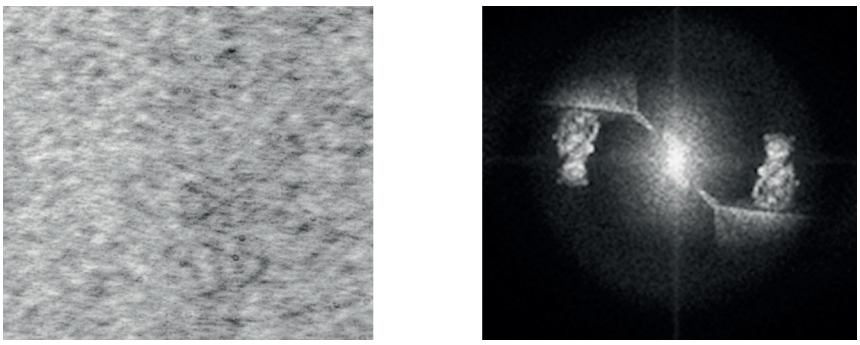

Fig. 3. Hologram and reconstructed images during its digitization with lens 40×.

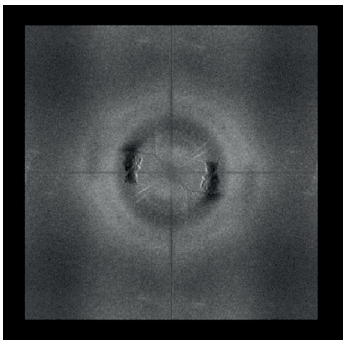

Fig. 4. Amplitude of the correcting aperture function.

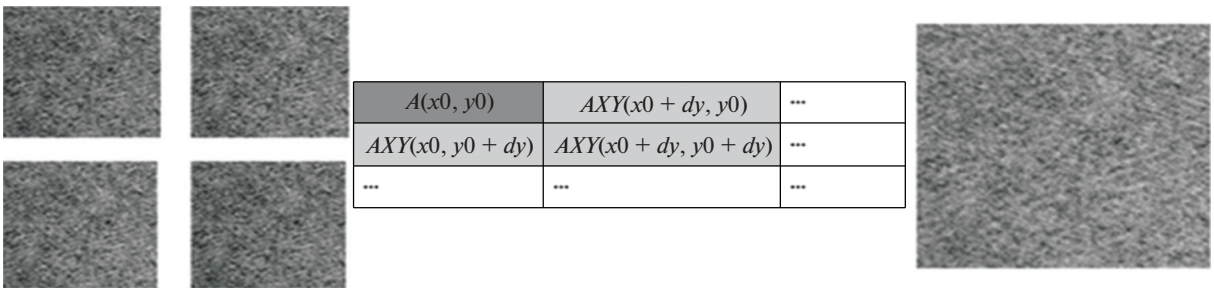

Fig. 5. Synthesis of a digital hologram with subpixel shifts.

the spectra due to the linearity of the Fourier transform, one can obtain a corrective aperture function (Fig. 4), which transforms the aperture function of the 8× lens into the aperture function of the 40× lens.

Restoring Holograms When Digitizing with an Insufficient Resolution Lens

Figure 5 shows the process of synthesizing a high-resolution image from low-resolution images obtained at various subpixel shifts [16, 17]. Four low-resolution digitized images of the hologram obtained with spatial shifts are shown on the left in Fig. 5 (from left to right and from top to bottom 0, dx, dy, and dx and dy, respectively). A diagram of the formation of a synthesized hologram imageis is shown in the center. A high-resolution image of a hologram is shown on the right in Fig. 5.

When synthesizing holograms with a lens magnification of eight times (8×), a subpixel shift (dx) of 900 nm was used; a subpixel shift of 460 nm was used for a lens magnification of 20×. After element-wise division of the spectra of the generated holograms into the spectrum of the aperture function (see Fig. 4), one can obtain reconstructed images (Fig. 6).

Figures 6a–6c show the results of reconstruction of digital holograms recorded by lenses 8×, 20×, and 40×. Consequently, it is possible to satisfactorily restore the image only from a hologram digitized

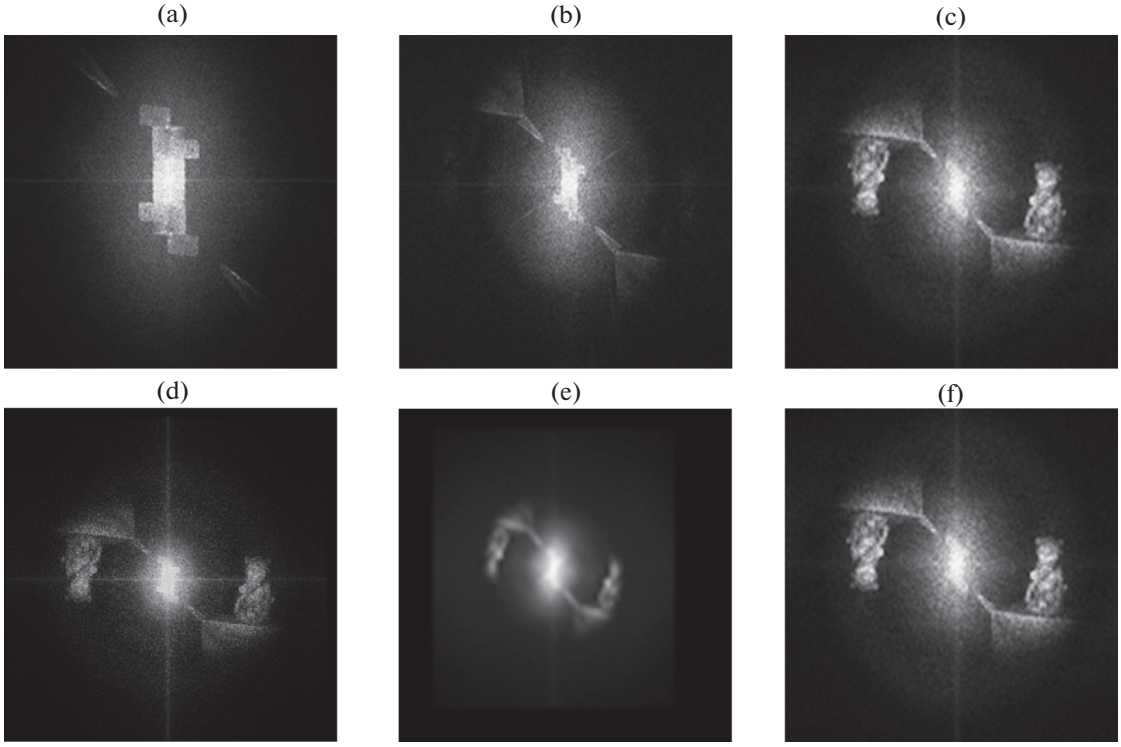

Fig. 6. Reconstructed part of the image from holograms obtained from four holograms with subpixel shifts at an angle of 30° between interfering beams recorded by lenses 8×, 20×, and 40× without subpixel shifts (*a*—c) and with subpixel shifts (d—f).

with a 40× lens. Figures 6d–6f show the reconstructed images after element-wise division of the spectrum of the generated holograms obtained with 8× and 20× lenses into the spectrum of the aperture function (see Fig. 4). It can be seen that the quality of the reconstructed images is comparable to the quality of the image recorded by the 40× lens. Thus, it is shown that using a lens with a lower resolution allows one to obtain qualitatively the same results as when using lenses with a higher resolution, if the aperture function is determined correctly. Accurate calibration requires a more accurate acquisition of the aperture function.

CONCLUSIONS

The article discusses the problem of increasing the spatial resolution of optical microscopes using a subpixel shift. It is shown that, due to the proposed approach, the use of lenses with low spatial resolution (8×) can give the same results as the use of lenses with high resolution (20× or 40×). This allows one to change the spatial resolution of the optical microscope without changing lenses.

Based on the results, we consider it appropriate to introduce a description of the aperture function into the lens passport. This will make it possible to create single-lens optical microscopy systems with a resolution that is not inferior to more complex and expensive lenses.

Some disadvantages include the need to introduce an additional device to introduce a subpixel spatial shift. However, lower magnification lenses have a larger field of view than higher magnification lenses. Note that lenses with higher magnification have a more complex design and, accordingly, they are more expensive. In addition, the ability to eliminate the need to have a set of lenses with different magnifications will allow the creation of simpler optical microscopy devices.

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CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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