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Obtaining High-resolution Images with a Large Field of View in Optical Microscopy

Ekaterina V. Andryushchenko
Department of Data Acquisition and Processing Systems
Novosibirsk State Technical University
Novosibirsk, Russia
andrushenkokv@mail.ru

Vladimir I. Guzhov
Department of Data Acquisition and Processing Systems
Novosibirsk State Technical University
Novosibirsk, Russia
vigguzhov@gmail.com

***Abstract*—Optical microscopy systems are widely used in various fields such as biology, medicine, and industry. The spatial resolution of a microscope is determined by the set of micro-lenses used. Due to their design features, there is an inverse dependence: the largest field of view is obtained when using a low magnification, low resolution objective lens. At the same time, lenses with lower resolution are cheaper due to the simplicity of manufacturing. Therefore, increasing the spatial resolution with such lenses is quite a promising direction of work. The aim of the paper is to improve the spatial resolution in optical microscopy by using objective lenses with large field of view. The enhancement of spatial resolution is accomplished using an analytical expression for the spectrum of discrete signals obtained using subpixel shifts. In this case, the spectrum of the function 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 multiplier depending on the chosen aperture. This multiplier is called the aperture function. The aperture function is determined by the type of lens used and can be its passport value. The paper shows an experimental method for calibrating a lens (obtaining its aperture function) with a low resolution (10X) based on images acquired with lenses with a high resolution (100X).**

Keywords—microscopy, discretization, Fourier transform, spectrum, super-resolution, subpixel shift

I. INTRODUCTION

In optical devices, it is necessary to confine light beams, which is accomplished by using aperture diaphragms. This leads to a reduction in the ultimate spatial resolution. Resolution is determined by the ability of an optical system to image two closely spaced point objects separately. This resolution is determined by the Rayleigh criterion:

$$R = 0{,}61\frac{\lambda}{NA^{obj}} \quad (1)$$

where NA^{obj} - numerical aperture, which depends on the design of the micro lens; λ - wavelength of the illumination source. In practice, at a wavelength of about 600 nm, the spatial resolution of optical microscopes does not exceed 200 nm [1], [2].

The magnification factor and numerical aperture are always indicated on the objective lens (Fig.1). In modern lenses with the same magnification factor, the numerical aperture is smaller.

Fig. 1. Labeling of used micro lenses.

The larger the numerical aperture of the objective lens, the smaller the field of view. The following table shows the maximum field of view as well as the resolution of the lenses at wavelength $\lambda = 0{,}6$ μm in the modified microscope.

TABLE I. CHARACTERISTICS OF THE LENSES USED

Lens magnification	Numerical aperture NA^{obj}	Field of view (mm)	Necessary resolution $R = 0{,}61\frac{\lambda}{NA^{obj}}$
10x	0,3	2,019	1,22 μm
100x (immersion)	1,3	0,228	0,282 μm

The paper considers increasing the spatial resolution of lenses by means of subpixel shifts, i.e. shifts by an amount smaller than one element of resolution.

The general formulation of the problem of increasing spatial resolution in optical systems is described in [3]-[7]. Fig.2 shows the scheme of registration of a one-dimensional signal when it is scanned by a low-resolution aperture. Here n is the number of low-resolution image elements, l is the number of high-resolution elements falling into the integrating aperture I_i, $i=0...n$, nl is the number of elements in the high-resolution image.

If as a result of measurements we obtain a set of low-resolution values I_i, shifted with respect to each other by some value smaller than the size of the integrated aperture, we can determine high-resolution elements x_i.

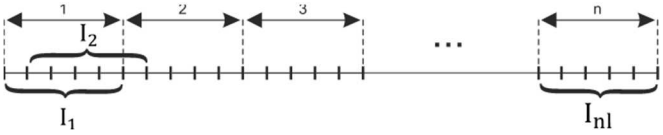

Fig. 2.Scheme of one-dimensional signal registration using subpixel shifts.

Further, using mathematical processing, super-resolution can be achieved when a higher resolution image is obtained from a series of low-resolution image frames of the same object [8].

In [9], a process for producing high-resolution images from low-resolution images obtained using sub-pixel scanning based on computer modeling is shown. The method is based on image formation by adding pixels obtained by subpixel shifts (Fig. 3).

A(x0,y0)	AXY(x0+dx,y0)	AXY(x0+2dx, y0)	AXY(x0+3dx, y0)	A(x1, y0)	...
AXY(x0, y0+dy)	AXY(x0+dx,y0+dy)	AXY(x0+2dx,y0+dy)	AXY(x0+3dx,y0+dy)	AXY(x1, y0+dy)	...
AXY(x0, y0+2dy)	AXY(x0+dx,y0+2dy)	AXY(x0+2dx,y0+2dy)	AXY(x0+3dx,y0+2dy)	AX(x1, y0+2dy)	...
AXY(x0, y0+3dy)	AXY(x0+dx,y0+3dy)	AXY(x0+2dx,y0+3dy)	AXY(x0+3dx,y0+3dy)	AX(x1, y0+3dy)	...
A(x0, y1)	AX(x0+dx,y1)	AX(x0+2dx,y1)	AX(x0+3dx,y1)	A(x1, y1)	...
...	...		...		...

Fig. 3. Subpixel image generation method for increasing the image resolution by a 5 times factor.

In the table, dark color shows the original pixels of the low-resolution image, gray color shows the pixels obtained by successive subpixel shifts.

In [10] it is shown that in this case the spectrum of the generated image will be determined by the following expression:

$$F_{\tau,\Delta x}(\omega) = \left(\left[\Im(f(x)) \otimes sinc\left(\frac{\omega N}{2}\right)\right] \cdot \Im(rect_\tau(x))\right) \quad (2)$$

where $\left[\Im(f(x)) \otimes sinc\left(\frac{\omega N}{2}\right)\right]$ - discrete Fourier image of the original function, $\Im(rect_\tau(x))$ - Fourier image of the used aperture (aperture function).

In this expression the type of function is unknown $\Im(rect_\tau(x))$.

From expression (2) we can see that if the Fourier image of the generated image is divided by the Fourier image of the high-resolution image, we obtain the view of this function. If the view $\Im(rect_\tau(x))$ can be obtained in any way, the Fourier image of the high-resolution image can be obtained from expression (2). By taking the inverse Fourier transform from it we obtain the original one.

The purpose of this paper is to experimentally obtain a high-resolution image with an increased field of view from a series of low-resolution images shifted by subpixel value.

II. Modification of Optical Microscope for Realization of Subpixel Shift

To realize the method of subpixel scanning, a modification of the metallographic aggregate microscope METAM-P1 [11] was carried out. A plane-parallel scanning piezo stage Ratis XYZ_(H) [12] was used as a slide. The use of this stage allowed to provide a range of motion in the XY region - 100 mm with a minimum motion step - 1 nm. For image sampling, a Canon EOS M50 serial digital mirrorless camera with an APS-C CMOS sensor with a resolution of 24.1 megapixels (6000x4000 pixels) was used. The sensor measures 22.3 x 14.9 mm and the pixel size in the sensor is 3.7 μm. The camera is connected to a computer via a USB cable.

The appearance of the modified microscope is shown in Fig.4.

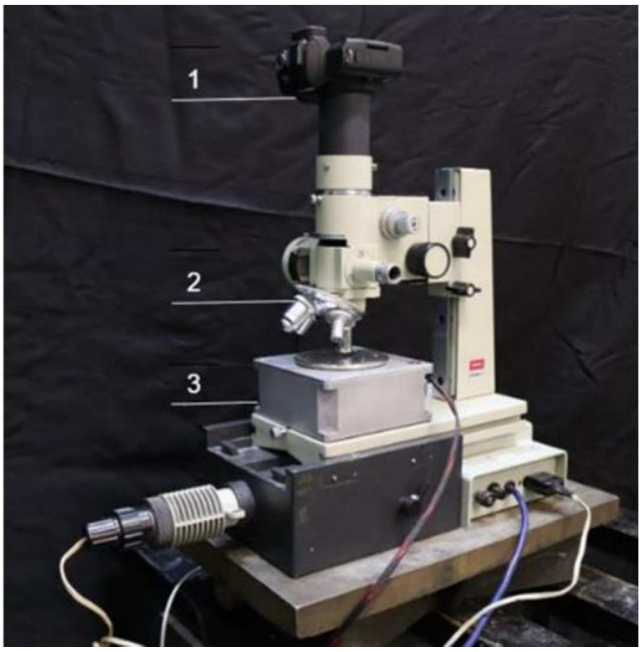

Fig. 4. Modified microscope (1 - digital camera, 2 - optical microscope with a set of micro lenses, 3 - automated slide).

Fig.5 shows the process of synthesizing a high-resolution image from low-resolution images obtained by successive subpixel shifts [9]. In this case, to increase the resolution by a factor of 5, it is necessary to make 24 steps.

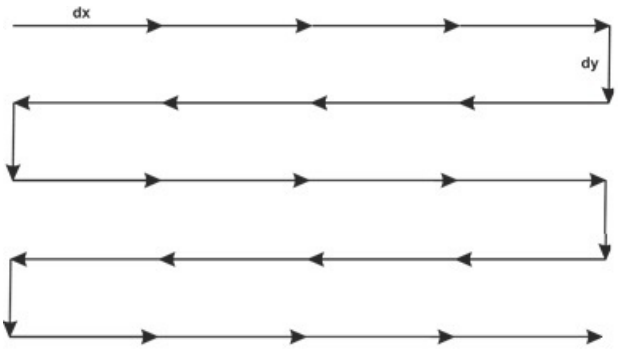

Fig. 5. Setting of spatial shifts with the shift value equal to half of the image resolution to increase the resolution by 5 times.

With the help of the modified microscope, it is possible to provide a subpixel shift of the object with possible steps at displacement: 5 μm, 1 μm, 500 nm, 100 nm, 10 nm, 1 nm.

III. Obtaining a High-Resolution Image with an Enlarged Field of View

To determine the aperture function, test images are needed. Images of microflora from oral mucosal swab were used as such images.

Fig.6 shows the comparison of the field of view for 10X and 100X lenses.

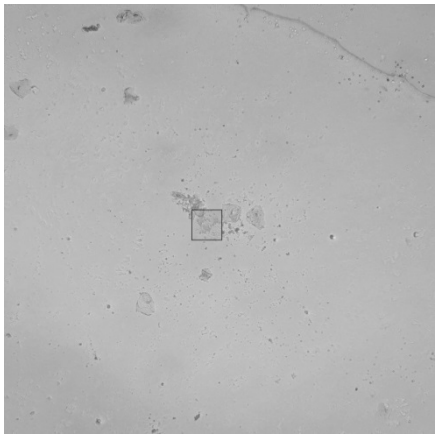

Fig. 6. Field of view of 10X and 100X lens.

Table I shows that the resolution of the 10X lens is 1.220 μm and the 100X lens is 282 nm.

With the 10X objective lens, 25 images (Fig.7) of the object were scanned with a subpixel shift of 244 nm, which were then combined according to the scheme shown in Fig. 3. The size of the image area was chosen so that it coincided with the field of view of the 100X objective.

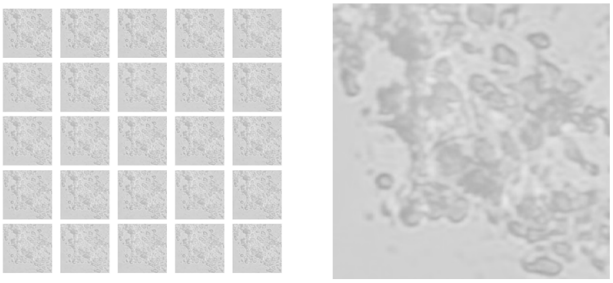

Fig. 7. Subpixel image generation with 10X lens, left - 25 digitized low-resolution images acquired with spatial shifts; right - generated image.

The size of the generated image is 2048x2048 pixels.

With a 100X lens, the same field was scanned with the same point size (2048x2048). After Fourier transform, the Fourier image of the generated image (10X) was pointwise divided by the Fourier image of the image scanned with 100X lens. Fig.8 shows the resulting aperture function.

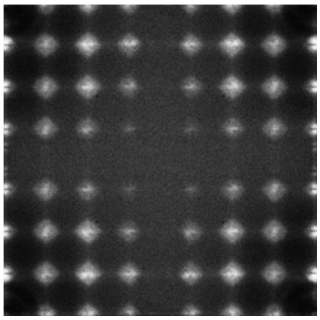

Fig. 8.Aperture function corresponding to 10X and 100X lenses.

The aperture function was also calculated for a number of other specimens. Its appearance depends only on the lenses used. The averaged aperture function from several specimens was further increased by a factor of 4. Then, following the scheme shown in Fig.3 using subpixel shifts of 244 nm, a new image of the object was generated with a 10X lens (Fig.9). In this case, the size of the field of view is twice as large as the field that can be recorded with a 100X lens.

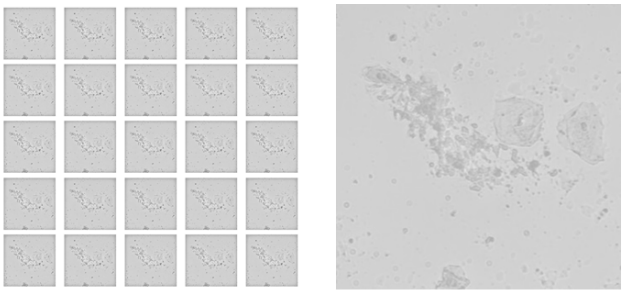

Fig. 9. Subpixel image generation with 10X lens.

The size of the generated image is 8192x8192 pixels.

After dividing the Fourier image of the generated image by the enlarged aperture function, the Fourier image of the original field was obtained. After the inverse Fourier transform, the full field image was obtained (Fig.10). The image has the same resolution as the 100X lens and the field of view is 2 times larger than at 100X.

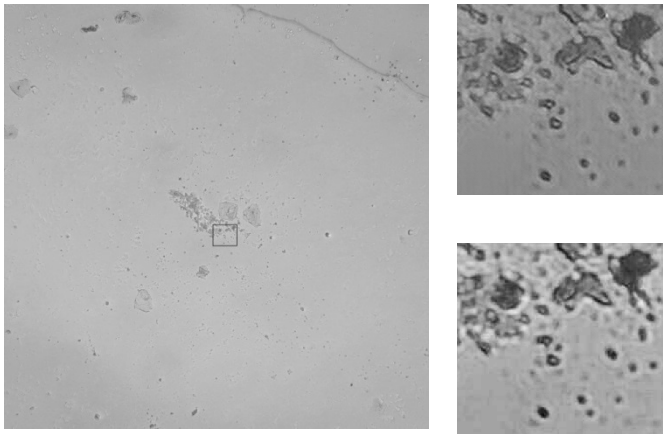

Fig. 10. Left: Image reconstructed from four 244 nm subpixel shifts at 10X magnification, right: two images at different resolutions, top - selected area in the reconstructed image, bottom - the same area of the object scanned with a 10X lens.

Comparison of a part of the field of view of the reconstructed image showed complete identity with the image of the same area obtained with the 100X lens. At that, the size of the field of view of the reconstructed image is twice as large as the field that can be fixed with the 100X lens (Fig.11).

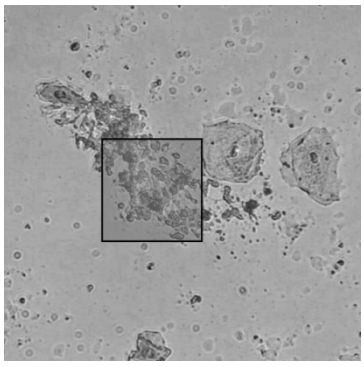

Fig. 11. Comparison of the field of view of the reconstructed image with the original image obtained with the 100X lens.

IV. Conclusions

In this article we considered the problem of reconstruction from a series of 25 images obtained by moving the objective stage at 244 nm with a 10X lens of an image with a resolution of 282 nm. Thus, if the aperture function for a series of lenses is determined, it is not necessary to use lenses with a higher resolution (100X). In the article, 10X and 100X lenses were used, but the same procedure is possible for lenses with other resolutions.

The advantage of this approach is that it is possible to use an extended field of view with low magnification lenses, with the same resolution as high resolution lenses. This makes it possible to avoid using expensive high-resolution lenses altogether.

A disadvantage of the method is the need to use an additional device to perform subpixel shifts. However, in many cases the use of subpixel shifts is preferable to the use of complex lenses with high resolution and small field of view.

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