# Image Resolution Enhancement Algorithm for Different Pairs of Optical Microscope Lenses

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***Abstract*—In the visible range, the maximum resolution of modern optical microscopes is limited by the diffraction limit. To study objects of smaller sizes, other types of microscopy are used (but these are rather complicated and expensive systems) or methods of program image processing. This paper discusses a program processing method using synthesized aperture techniques. Based on it, the paper shows an experimental method for obtaining high-resolution images from low-resolution images. The images are obtained using optical microscope lenses with different resolutions (10X, 40X and 100X). As a result of processing the obtained images, aperture functions are calculated for each of the considered pairs of lenses. With the help of these aperture functions it is further possible to obtain high-resolution images from low-resolution images, without the use of expensive equipment, or more difficult to operate lenses (e.g., immersion lenses). The developed method will overcome the diffraction limit of optical microscopy, and achieve a resolution that is determined by a given spatial shift. Thus, the quality of optical microscopy measurements will become commensurate with other, more expensive and high-precision types of microscopy, while remaining more accessible and cheaper.**

***Keywords—subpixel shift, spatial resolution, synthesized aperture, optical microscopy, generalized functions***

## I. Introduction

This paper presents a method for analyzing Fourier images obtained by averaging regions (apertures) of finite size. The method was written in detail in the articles [1], [2], [3], [4].

Its essence can be described as follows. Discretization is performed by measuring the signal using a limited set of sensors with some aperture (the area over which averaging takes place) of finite size. (The scheme of registration of one-dimensional signal is presented in Fig.1.). The numerical values of the image samples are obtained by measuring the signal using sensors with some finite aperture (the area over which the values are averaged). In optical systems, the type of aperture depends on the lens used and determines its resolution capability. In this paper, the "aperture function" is the Fourier image of the aperture used.

In the Fig.1. $n$ – the number of low-resolution image elements, $l$ – the number of high-resolution elements falling into the integrable aperture $I_i$, $i=0...n$, $nl$ – the number of elements in the high-resolution image.

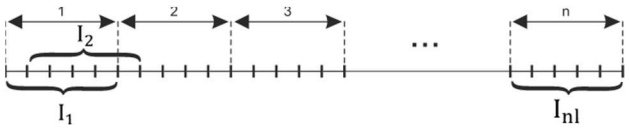


Fig. 1. Example of one-dimensional signal recording.

Then mathematical processing of the subpixel shift images is performed. From these images, by solving the system of linear equations (1), we obtain the required high-resolution components [3], [5], [6], [7], [8]:

$$\begin{aligned} &x_1 + x_2 + \dots x_i = I_1 \\ &x_2 + x_3 + \dots x_{i+1} = I_2 \\ &\dots \\ &x_{(n-1)l+1} + x_{(n-1)l+2} + \dots x_{nl} = I_{nl} \end{aligned} \quad (1)$$

where $x_i$ – high-resolution element.

Fig. 2. shows an example of two-dimensional subpixel scanning. A series of low-resolution images is obtained by successively shifting the same object [5], [9]. The 4 frames required to increase the resolution by a factor of 2 are highlighted in red line. At the same time, 25 frames are needed for resolution increases by a factor of 5.

| | | | | | |
|---|---|---|---|---|---|
| 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. 2. Procedure for acquiring a series of images to increase the image resolution by a factor of 2 and 5.

## II. Modificated Installation Based on Optical Microscope

A system based on an optical microscope [10] was developed, which, together with the developed software, allows increasing the spatial resolution of microscopic images. In particular, the microscope was modified with a "Ratis" scanning stage [11], which provides the ability to shift the object by nanometers. And also, the microscope was completed with a digital camera Canon EOS M50, which allows discretization of images. More details about the equipment were described in [2].

The design of the modernized system is presented in Fig. 3.

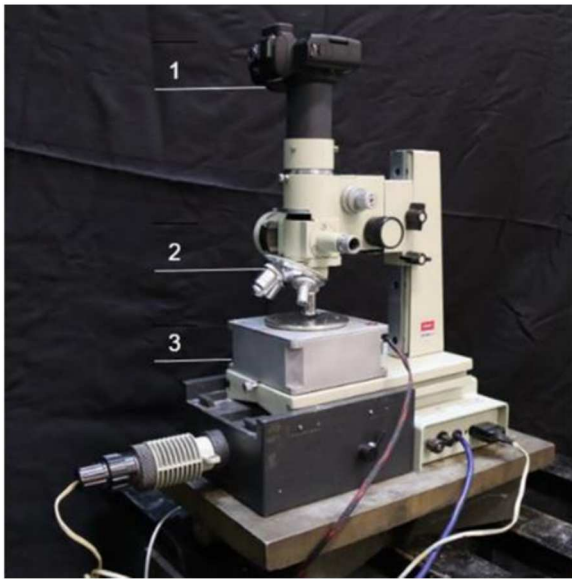


Fig. 3. Modified system based on optical microscope (1 - digital camera, 2 - microscope, 3 - piezoelectric stage).

The modified microscope allows a series of frames to be acquired with a minimum step size of 1 nm.

The magnification coefficient and numerical aperture of the lenses used in this paper are shown in Fig.4 and Table I.


Fig. 4. Marking of used lenses.

TABLE I. LENS SPECIFICATIONS

| Lens magnification | Numerical aperture $NA^{obj}$ | Field of view | Necessary resolution $R = 0,61\frac{\lambda}{NA^{obj}}$ |
|---|---|---|---|
| 10x | 0.3 | 2.019 mm | 1.22 µm |
| 40x | 0.65 | 0.541 mm | 0.563 µm |
| 100x (immersion) | 1.3 | 0.228 mm | 0.282 µm |

From Table I we see that the required resolution for a 100X lens (images at 100X are used as a reference) is 0.282. To get the resolution closest to 100X with the 10X lens, we divide its resolution of 1.22 by 5. We get the necessary subpixel scanning step *dx*=0.244. For the 40X lens, we divide its resolution 0.563 by 2 and get *dx*=0.282.

Thus, having determined the number of images and the step of scanning for each pair of lenses, it is possible to obtain an image comparable in quality with the reference one.

## III. ENHANCING THE RESOLUTION OF THE SAME IMAGE USING DIFFERENT PAIRS OF LENSES

The method consists of calculating the aperture function for a selected pair of lenses (one with low resolution and one with high resolution) [9]. This paper evaluates the effectiveness of the method for different pairs of lenses of a modified optical microscope. Evaluation of the effectiveness of the method consists in a visual comparison of the quality of improved (reconstructed) and reference (with a 100X lens) images.

The microflora image taken with three lenses was used in this work. Digitized images of the same area of the object received different quality and field size. To understand the differences between the lenses used, the ratio of the fields of view of the 10X, 40X and 100X lenses are shown in Fig.5.

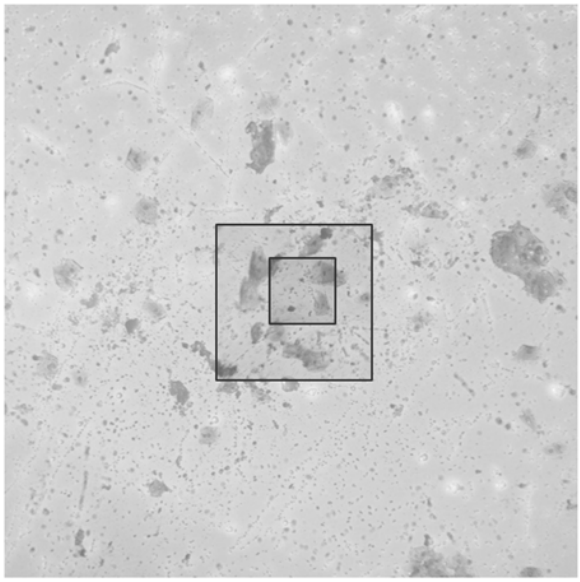

Fig. 5. Field of view of 10X, 40X and 100X lens.

### A. High-Resolution Image Generation Using 40X and 100X Magnification Lenses

Fig.6. shows a schematic of the 3 consecutive spatial shifts (steps) required to increase the image resolution by a factor of 2 [2]. The amount of shift is equal to half the resolution of the image.


Fig. 6. Subpixel scanning trajectory to increase the image resolution by a factor of 2.

Four images (Fig. 7. left) were acquired according to the 2-x image quality enhancement algorithm shown in Fig. 2. The object was scanned using a 40X objective lens sequentially with a step size *dx*=*dy*=282 nm. Then, these images were combined as shown in Fig.6. Thus, a generated image was obtained (Fig.7. right).

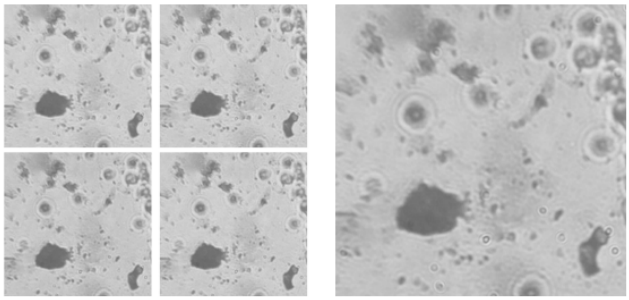

Fig. 7. Image registration using a 40X lens, left - 4 low-resolution images captured with subpixel shift; right - image formed from them.

A similar section of the object was captured using a 100X immersion lens. Then we obtain Fourier images of both images (formed at 40X and the original one at 100X). Next, we divide these images [2]. Thus, we obtain the aperture function for this pair of lenses (Fig. 8).

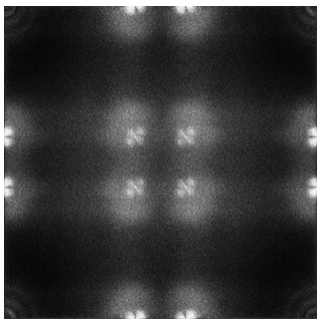

Fig. 8. Aperture function for a pair of 40X and 100X lenses.

Further, if we divide the Fourier image of the generated image (40X - Fig.7. right) by the aperture function (Fig.8.), we obtain the Fourier image of the original image. As a result, after the inverse Fourier transform, we obtain the original high-resolution image (Fig. 9) [2].

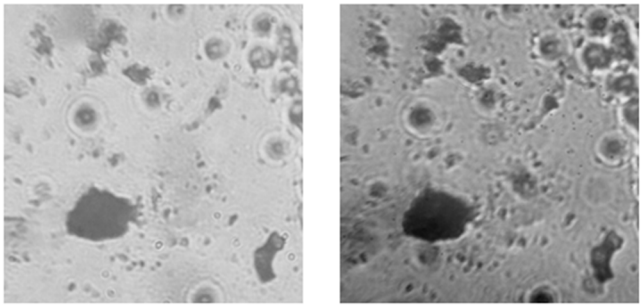

Fig. 9. Comparison of original and resulting images, left – original image acquired with a 40X lens; right – reconstructed high-resolution image at 3 subpixel shifts.

### *B. High-Resolution Image Generation Using 10X and 100X Magnification Lenses*

It takes 24 steps to increase the resolution of the image by a factor of 5. This coefficient is calculated for this pair of lenses.

Fig.10. shows the spatial shift scheme for this case.

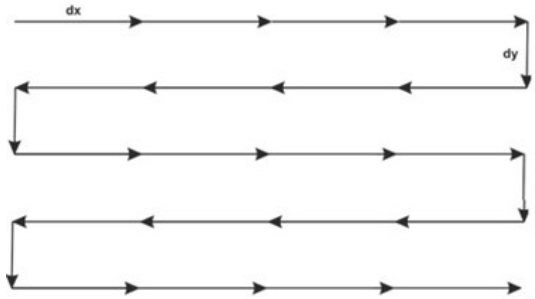


Fig. 10. Subpixel scanning trajectory to increase the image resolution by a factor of 5.

25 images (Fig. 11. left) were acquired according to the 5-x image quality enhancement algorithm shown in Fig. 2. The object was scanned using a 10X objective lens sequentially with a step size $dx=dy=244$ nm. Then, these images were combined as shown in Fig.10. Thus, a generated image was obtained (Fig.11. right).

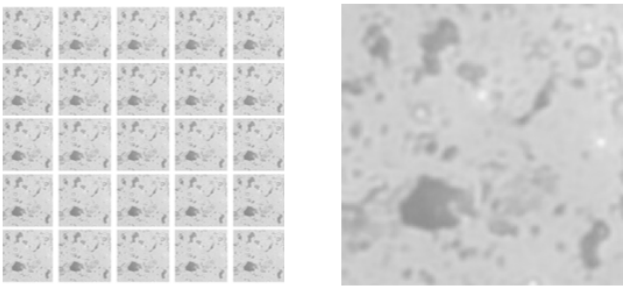

Fig. 11. Image registration using a 10X lens, left - 25 low-resolution images captured with subpixel shift; right - image formed from them.

Then, the steps from Section A are repeated to calculate the aperture function for the 10X and 100X lens pair (Fig. 12.).

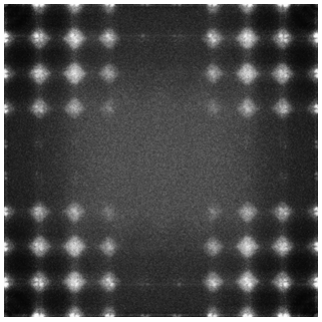

Fig. 12. Aperture function for a pair of 10X and 100X lenses.

Next, the algorithm from the previous section is repeated and the original high-resolution image is obtained for a pair of 10X and 100X lenses (Fig. 13).

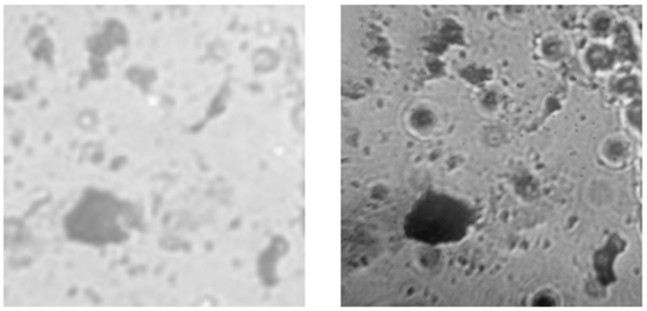

Fig. 13. Comparison of original and resulting images, left – original image acquired with a 10X lens; right – reconstructed high-resolution image at 24 subpixel shifts.

## IV. Results

Fig. 14. Shows plots of the original images obtained with 10X, 40X and 100X lenses. It can be seen that when using 10X lens the image quality is noticeably lower than 100X. At 40X the image quality is already closer to the image at 100X, but some of the smallest elements are distorted.

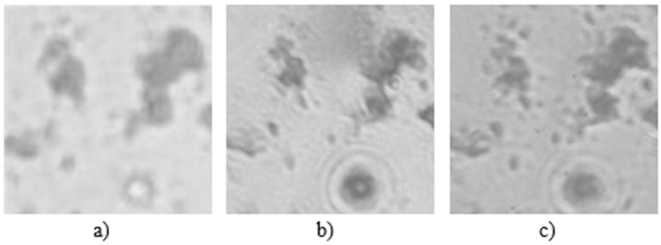


Fig. 14. Plots of the original images acquired with 10X, 40X and 100X lenses without processing (a-c).

Fig. 15 shows the reconstructed images after element-by-element division of the spectrum of the generated images obtained with the 10X and 40X lenses into the spectra of the corresponding aperture functions. It can be seen that the quality of the reconstructed images is comparable to that of the images obtained with the 100X lens.

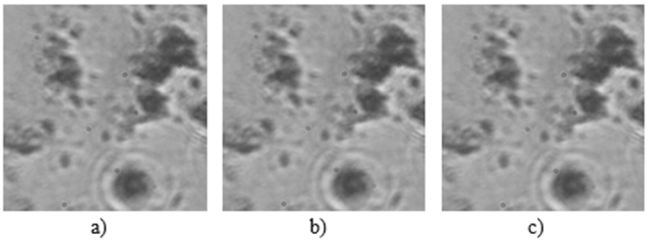


Fig. 15. Plots of reconstructed images obtained with subpixel shift processing recorded with 10X, 40X and 100X lenses (a-c).

Thus, it is shown that using a lower resolution lens produces qualitatively the same results as those obtained with higher resolution lenses if the aperture function is defined correctly.

## V. Conclusion

In this paper, high quality images were experimentally acquired using optical microscope lenses with different resolutions (10X, 40X and 100X). As a result of the processing, aperture functions were calculated for each of the considered pairs of lenses. Using these aperture functions, it is then possible to obtain high-resolution images from low-resolution images without using expensive equipment or more difficult to operate lenses (e.g., immersion lenses).

The developed method will overcome the diffraction limit of optical microscopy and achieve a resolution that is determined by a given spatial shift. Thus, the quality of optical microscopy measurements will become commensurate with other, more expensive and high-precision types of microscopy, while remaining more accessible and cheaper.

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