Resolution-improved digital refocusing microscope for microstructure measurement

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Abstract

In general, it is quite difficult to measure three dimensional (3D) geometric information by using the optical microscope, since the spatial resolution is limited by the optical diffraction and the depth of field is very shallow. Therefore, the image stacking methods using the focal plane scanning or the stereoscopic imaging are typically employed to obtain depth information of an object to be measured. However, these methods are not suitable for industrial applications due to the complicated system and a large number of required images. Hence, we proposed a microscopic imaging method with the expanded depth of field and the improved-resolution. In this study, the experimental apparatus was developed, in which the microlens array and the optical path control unit were introduced into the optical imaging system. Then, the digital refocusing and the resolution improving based on the multi-image reconstruction were implemented to verify the proposed method fundamentally. A microscope test target inclined with respect to the object plane of the imaging was employed in a series of experiments for the 3D structures with the certain geometric properties. The resulting image was not only in-focus in all microscopic field but also improved in the spatial resolution compared with the conventional digital refocusing microscopy. Furthermore, 3D geometric information was extracted from the stacked images by using the shape from focus method.

1 Resolution-improved digital refocusing microscope

This study aims the establishment of the geometric structure measurement in the nano-micro region using the optical microscopic images. In the conventional optical microscopy, it is difficult to obtain 3D geometric information, since the depth of field of the optical microscope is extremely shallow. For example, the laser scanning confocal microscope is capable of the 3D micro-geometric measurement, but it is expensive and time-consuming for the practical applications. Therefore, we proposed the resolution-improved digital refocusing microscope for high-speed measurement.

1.1 Digital refocusing microscopy

In recent years, the digital refocusing, which is a kind of computer graphics and image stacking methods, has been applied for the fluorescent microscopic imaging to expand the depth of field [1]. The digital refocusing is based on the inverse ray tracing from the image plane to the intermediate image plane by using the light field recorded by the CCD image sensor with the microlens array [2].

1.2 Improving the resolution of the refocused images

The problem still remains in the digital refocusing microscopy, however, that the spatial resolution is limited by the pitch of the microlens used in the digital refocusing. The lateral resolution was drastically decreased, since this limit is much larger than the wavelength of the visible light. In order to compensate for the decrease in the spatial resolution of the digital refocusing microscopy, the resolution improving method based on the imaging path control [3] is useful. This method is based on the active subpixel shift of the imaging path in case of the large spatial sampling interval, which corresponds to the pitch of the microlens. The schematic optical system of the proposed method is shown in Figure 1. The imaging path is shifted by rotating a refraction glass in X and Y directions. This method makes it possible to obtain high resolution 3D geometric information with an one-direction-shot microscopic imaging. Furthermore, the wide-field all-in-focus image, which is also acquired with this method, is available and applicable for the high-throughput structure visualization and the review inspections in the micro-device industry.

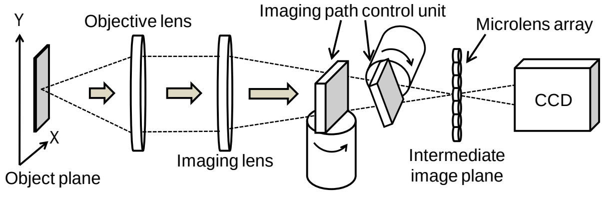

Figure 1: Optical system of the resolution-improved digital refocusing microscope

2 Experiment for microstructure measurement

In this section, the experimental verification of the proposed method is described. The developed apparatus is shown in Figure 2. This apparatus has two imaging path control units which consist of a refraction glass and a galvano scanner. A microscope test target inclined with respect to the object plane of the imaging was employed as a specimen in a series of experiments for the 3D structures with the certain geometric properties. The condition of the experiments is shown in Table 1.

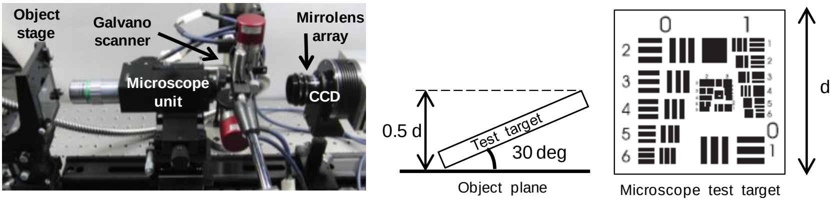

Figure 2: Experimental apparatus and specimen with microstructure

Table1: Condition of experiments

Microscope magnification	20
Numerical aperture of objective lens	0.28
Pixel size of CCD camera	6.5 μm
Pitch of microlens	100 μm
Number of images for resolution improvement	5×5 = 25 images
Line pitch distribution in test target	4.4 μm to 16 μm
Inclined angle of test target	30 deg

2.1 Expanded depth of field and improved resolution

In the experiments, multiple images through the microlens array with respect to the subpixel-shifted imaging paths were recorded by using the developed apparatus. Then, the digital refocus processing and resolution improving processing were implemented. The resulting images are shown in Figure 3. In the conventional microscopic image (a), the center row in the imaging area was partially in-focus because of the shallow depth of field. Although the digital refocused image (b) was all-in-focus, the fine structure was not resolved due to the decreased spatial resolution. On the other hand, the image aquired with the proposed method (c) was not only all-in-focus but also improved in the spatial resolution.

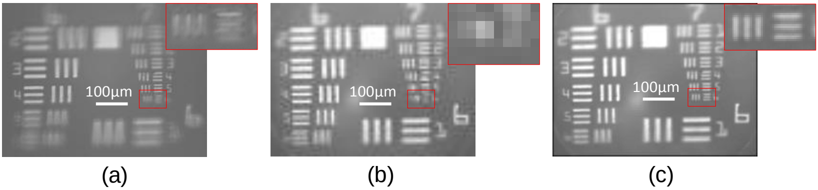

Figure 3: (a) conventional microscope image, (b) refocused all-in-focus image, (c) resolution-improved all-in-focus image

2.2 Geometric information extraction using shape from focus

In order to extract 3D geometric information of the fine structure of the specimen, the shape from focus method based on the wavelet transform [4] was applied to the resolution-improved focal stack obtained by proposed method. The result of the shape from focus including the texture with the all-in-focus image is visualized in Figure 4. Extracted 3D information approximately corresponded to the inclined specimen.

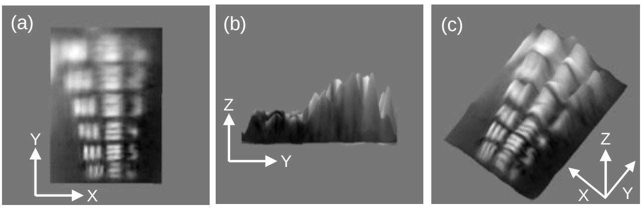

Figure 4: (a) top view of the result of the shape from focus using the resolution-improved and refocused focal stack, (b) side view, (c) 3D view

3 Conclusion

Resolution-improved digital refocusing microscope was proposed for the geometric structure measurement in the nano-micro region using the optical microscopic images. The resolution-improved all-in-focus image and its 3D geometric information were experimentally obtained.

References:

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