Proc. of the 9th Intl. Conf. of Asian Society for Precision Engg. and Nanotechnology (ASPEN 2022)
15–18 November 2022, Singapore. Edited by Nai Mui Ling Sharon and A. Senthil Kumar
doi://10.3850/978-981-18-6021-8_OR-11-0305

High-resolution 3D imaging by structured illumination microscopy

Jong-Kyu Park[1], and Ki-Nam Joo[1,#]

1 Dept. of Photonic Engineering, Chosun University, 309 Pilmun-daero, Dong-gu, Gwangju, 61452, South Korea
Corresponding Author / Email: knjoo@chosun.ac.kr, TEL: +82-62-230-7235, FAX: +82-62-230-7440

KEYWORDS: High resolution, Structured illumination microscopy, 3D imaging

In this investigation, we describe the combined approach with SR-SIM and OS-SIM to obtain high-resolution 3D images of the industrial specimens. By using digital micromirror device (DMD), the spatial sinusoidal pattern is rotated and phase-shifted, imaged on a specimen and a CCD cameara. By adopting 2D Fourier transformation, each high-resolution image at the specific axial position is calculated, and the 3D imaging was reconstructed by the aid of the OS-SIM. We believe this combined approch of SIM can be successfully applied in semiconductor and display industries.

NOMENCLATURE

I_1 = image with the 0-phase shifted sinusoidal pattern
I_2 = image with the 2π/3-phase shifted sinusoidal pattern
I_3 = image with the 4π/3-phase shifted sinusoidal pattern
I_S = optical sectioning image

1. Introduction

Recently, the needs for measuring the minute 3D structures on the substrate are increasing in the fields of semiconductor and display industries. Because of the limited lateral resolution due to the Abbe diffraction limit, the conventional optical system cannot achieve the high-resolution imaging, and even the 3D imaging is another challenging issue in optical system with the lateral resolution beyond the diffraction limit. One of the candidates for this purpose is the structured illumination microscopy (SIM) because it has the capability to improve the lateral resolution in the 3D imaging.

Typically, SIM has been developed for observing biomedical samples based on fluorescent microscopic techniques to overcome the limitations of wide-field microscopy. Although widefield fluorescence microscopy is very useful as a sensitive method for detecting various proteins, it has the difficulty to provide the clear image of a sample when the image blurring caused by the light from out-of-focus regions is occurred. To prevent the image blurring, structured illumination microscopy uses a structured illumination pattern to restrict the out-of-focus light and enhances the fluorescent image quality of the sample. Typically, structured illumination microscopy has been developed as two categories; lateral resolution enhancement, so called super-resolution structured illumination microscopy (SR-SIM) [1] and 3D optical sectioning structured illumination microscopy (OS-SIM) [2]. SR-SIM enhances lateral resolution by collecting several patterned images and analyzing them in reciprocal space such as in Fourier domain. On the other hand, OS-SIM can provide 3D imaging of the sample by detecting the visibility of the illumination pattern.

SIM can also measure 3D surface profiles of various specimens such as semiconductor and display products without the florescent imaging. By using a sinusoidal amplitude grating, in this case, the spatial light pattern can be imaged to the surface of the specimen and obtained by an image sensor to reconstruct the high-resolution image. Similar to confocal scanning microscopy, furthermore, the specimen is axially scanned, and SIM can detect the position of the maximum visibility to find out the surface height of the specimen.

In this investigation, we describe the combined approach with SR-SIM and OS-SIM to obtain high-resolution 3D images of the industrial specimens. By using digital micromirror device (DMD), the spatial sinusoidal pattern is rotated and phase-shifted, imaged on a specimen and a CCD camera. By adopting 2D Fourier transformation, each high-resolution image at the specific axial position is calculated, and the 3D imaging was reconstructed by the aid of OS-SIM.

2. Structured illumination microscopy

2.1 Super resolution structured illumination microscopy

Super resolution structured illumination microscopy (SR-SIM) can enhance the lateral resolution by involving the illumination pattern to the sample. In this case, the spatial frequencies of the pattern and the specimen are mixed together, and they can be extended beyond the optical transfer function (OTF) determined by the diffraction limit as shown in Fig. 1. Based on the moiré effect between the sinusoidal illumination pattern and sample's structural distribution, the frequency information of the sample, which is beyond the support of the OTF, can be down-shifted and collected through the imaging system. By combining the spatial frequency contents of rotated images, the whole spatial frequency region is extended twice in linear SR-SIM, which indicates the lateral resolution of the reconstructed image is improved twice compared to that of the original image.

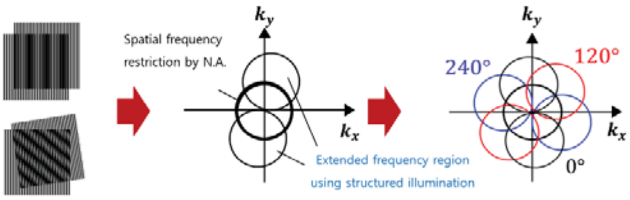

Fig. 1 Extended spatial frequency region by structed illumination.

2.2 Optical sectioning structured illumination microscopy

In optical sectioning structured illumination microscopy (OS-SIM), a specimen is illuminated by a sinusoidal pattern which enables to localize the measurement area and the 3D optical sectioning images can be obtained according to the axial scanning. At each axial position, the sinusoidal pattern is phase-shifted as an amount of 0, 2π/3 and 4π/3 to get images, I_1, I_2 and I_3, respectively. From these phase shifting images, the optical sectioning image or the contrast of the sinusoidal pattern (I_S) is calculated as a form of [2]

$$I_S = [(I_1 - I_2)^2 + (I_1 - I_3)^2 + (I_2 - I_3)^2]^{1/2} \qquad (1)$$

During axial scanning, the sectioning images are collected at every axial position and then the contrast curve at each pixel of images can be drawn with the axial position. In this case, the contrast peak position at each pixel means the best focus position of the surface of the specimen, which can reconstruct the 3D surface profile of the specimen.

2.3 Structured illumination microscopy for high resolution 3D imaging

Figure 2 shows the optical configuration of the structured illumination microscopy combined with SR-SIM and OS-SIM. A digital micromirror device (DMD) is used for generating the sinusoidal pattern for the illumination, and makes the pattern rotated and phase-shifted. A specimen is attached to the moving stage, and the contrast of the pattern is obtained along the axial positions. In SR-SIM, the high-resolution images are obtained by using 2D Fourier transformation to combine the spatial frequency contents while the height information of the specimen is calculated by the contrast peak detection of every pixel of the image in OS-SIM. At the final stage, the measurement results of two modes are combined to reconstruct the high-resolution 3D image of the specimen.

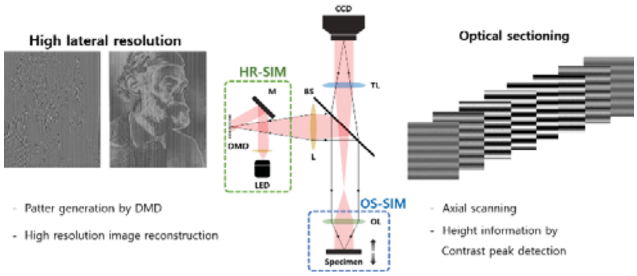

Fig. 2 Optical configuration of the structured illumination for high resolution 3D imaging; DMD, digital micromirror device; M, mirror; L, lens; BS, beam splitter; OL, objective lens; TL, tube lens.

3. Experimental results

Figure 3 shows the experimental results to measure a resolution target to verify the performance of the SIM. As shown in Fig. 3(a), the reconstructed image by SR-SIM was obtained with 9 rotated and phase shifted images, and the lines of the target was more obviously seen than the original image. On the other hand, the height information of the specimen was obtained by the contrast detection of the pattern in OS-SIM, and the step height of the specimen was calculated as approximately 0.4 μm as shown in Fig. 3(b).

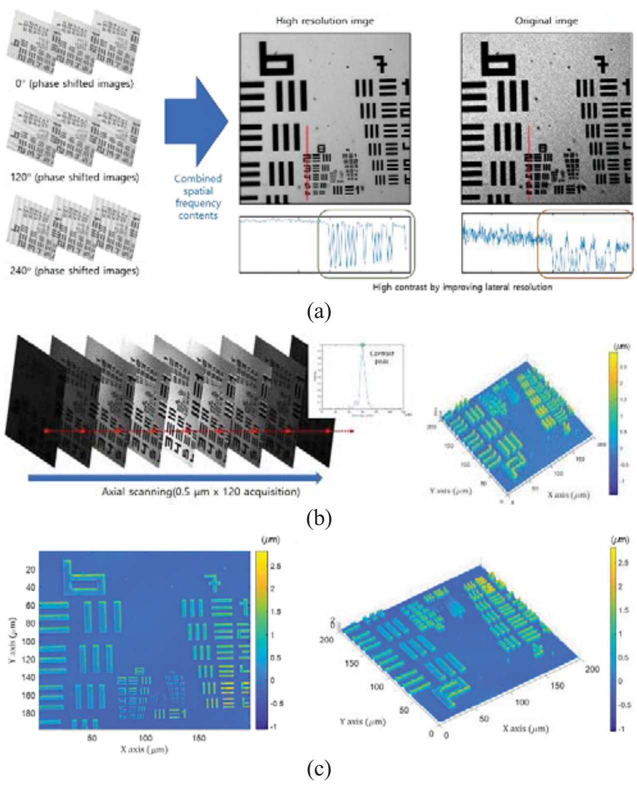

Fig. 3 Measurement results of (a) SR-SIM, (b) OS-SIM and (c) combined measurement results with (a) and (b).

Based on the height information of OS-SIM, the high-resolution 3D image of the specimen was reconstructed as shown in Fig. 3(c). As the result, the lateral resolution was improved as 1.3 times (0.7 μm) than the original image (1.0 μm), attributed to the relatively large period of sinusoidal illumination pattern. The axial resolution was less than 1.0 μm.

4. Conclusion

We demonstrated SIM for high resolution 3D imaging. By combining the operating principles of SR-SIM and OS-SIM, the spatial frequency region was extended and the contrast of the sinusoidal pattern was obtained. In the experiment, the resolution target was used and it was confirmed that the lateral resolution of the reconstructed image was improved with the 3D height information. We believe this combined approach of SIM can be successfully applied in semiconductor and display industries.

ACKNOWLEDGEMENT

This work was supported by the Ministry of Trade, Industry and Energy (20016563).

REFERENCES

1. Gustafsson, M. G. L., “Surpassing the lateral resolution limit by a factor of two using structured illumination microscopy,” J.Microsc., Vol. 198, No. 2, pp. 82-87, 2000.
2. Neil, M. A. A., Juškaitis, R. and Wilson, T., “Method of obtaining optical sectioning by using structured light in a conventional microscope,” Opt. Lett., Vol. 22, No. 24, pp. 1905–1907, 1997.