OPTICAL MICROSCOPY WITH IMPROVED RESOLUTION USING TWO-BEAM INTERFERENCE OF LOW-COHERENCE LIGHT

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Abstract:
In recent years, high-resolution microscopy using structured illumination has been practically applied for fluorescent bio-imaging. However, there is a large amount of speckle noise in reflected- and scattered-light images, because structured illumination is typically generated by laser-beam interference. Hence, this high-resolution imaging technique cannot be effectively used in industrial applications. In this study, we attempted to generate structured illumination using two-beam interference of low-coherence light, and to develop a high-resolution and low-speckle imaging technique for industrial applications. First, we constructed an optical system consisting of a Michelson interferometer configured in such a manner that it achieved zero optical path-length difference and allowed the interference fringes to be manipulated. Second, we observed a sample pattern of 0.2-μm-sized dots to confirm the generation of interference fringes under structured illumination. Then, we endeavored to observe other sample dotted patterns with several types of pitches. In one case, the pattern pitch of 0.4 μm was beyond the diffraction limit of 0.5 μm. In this experiment, we could obtain an image and observe the fringe patterns corresponding to features of low-coherence interference. The patterns show a moiré effect due to the combination of the periodic sample patterns and the structured illumination, and we confirmed that the moiré-pattern width corresponded to the coherence length of the light source. Finally, we reconstructed a resolution-improved image from an image stack resulting from spatial scanning of the generated structured illumination. As a result, the narrow pitch pattern of 0.4 μm was successfully resolved with relatively less speckle noise.

Keywords: Optical Microscopy, Structured Illumination, Two Beam Interference, Low-Coherence Light

1. INTRODUCTION

In recent years, high-resolution optical microscopy using structured illumination [1] has been developed and used in fluorescent bio-imaging applications [2,3,4,5]. Since structured illumination can be generated over the entire observation area, the technique enables high-speed and high-resolution imaging through parallel image processing. Therefore, it is expected to be applicable to not only fluorescent bio-imaging but also imaging in industrial fields. However, there are two critical problems for eliciting high performance. First, speckle noise affects the observed image, especially in industrial fields, because the structured illumination is typically generated by laser interference; since industrial imaging is based on coherent image formation, it is difficult to use structure illumination effectively. Although random speckle noise can be suppressed by using a laser beam homogenizer or by reconstructing from a series of images [6], suppressing noise from the observed sample pattern is difficult. In particular, speckle noise critically affects defect inspection of optically-transparent (or semi-transparent) substrate surfaces and causes artefacts that can raise false detections during reconstruction. Second, because structured-illumination microscopy theoretically requires several phase-shift images, there is a requirement for employing high-precision piezoelectric stages for determining the illumination phase shift value. Furthermore, prospective use in an industrial setting requires the consideration of special circumstances, such as vibration, under which conventional laboratory systems may not function properly.

In this study, we propose to use a super-luminescent diode (SLD) as a low-coherence light source instead of a laser, which is typically used for structured illumination. This allows for speckle noise to be suppressed because speckle noise is produced by highly-coherent light sources. When an observation is performed under environmental disturbance or by using a low-precision stage such as the commonly used stepping motor stage, phase shift value cannot be determined precisely. However, in such a situation, our proposed method can determine phase shift value from an observed image stack only, because low-coherence interference can be utilized to find the precise location of the interference point. Moreover, since highly specific instrumentation is not required, structured illumination using an SLD offers the same attractive features as optical microscopy — high-speed and low-cost observation.

2. STRUCTURED-ILLUMINATION MICROSCOPY

Structured illumination exploits the moiré effect between the fringe-shaped intensity distribution of the structured illumination and the spatial periodicity of the observed samples, which shifts high-frequency spatial information to a low-frequency signal such that the high-frequency information can be reconstructed through signal processing in order to realize sufficiently high resolution [7]. Figure 1 shows a schematic image of the moiré effect. When two high-frequency fringes are overlapped, a low-frequency fringe appears. Considering the distance between two neighboring points to be the peaks on a sinusoidal wave, two close points are considered to high frequency sinusoidal waves. The spatial frequency coordinate system is expressed by the k_x- and k_y-axes, which correspond to the x- and y-axes of the Cartesian coordinate system, respectively. Figure 2 is a conceptual diagram showing how high resolution is achieved

using the structured illumination. Here, the k_x- and k_y-axes designate the spatial-frequency coordinate system, and the area inside the circle indicates the frequency region resolvable with a microscope. Figure 2 (a) indicates that the frequency at a distance of k_1 from the origin can be resolved. Figure 2 (b) shows the shifting of the pass band due to the structured illumination microscopy and the moiré effect between the structured illumination and the sample. For example, shifting the pass band to a distance of k_2 from the origin yields a new resolution k_1+k_2 in the direction of k_2. However, the resolution is decreased to k_1-k_2 in the opposite direction, since this method only shifts the pass band and cannot expand it. When we illuminate the fringe pattern, which can be modulated in only one direction, we obtain an image with a resolution improved in only one direction. However, since we can obtain this improved resolution in any arbitrary direction, we need to apply the method repeatedly in various directions in order to obtain an image with high resolution in all directions. For example, we successively modulate the fringe pattern in three different directions, resulting in improved resolution in nearly every direction. However, we cannot extract a high-frequency component from an image generated in this way. Therefore, sequential images are generated and the requisite mathematical operations are performed. This results in a resolution twice as high as can be achieved using the conventional structured illumination microscopy method [8,9].

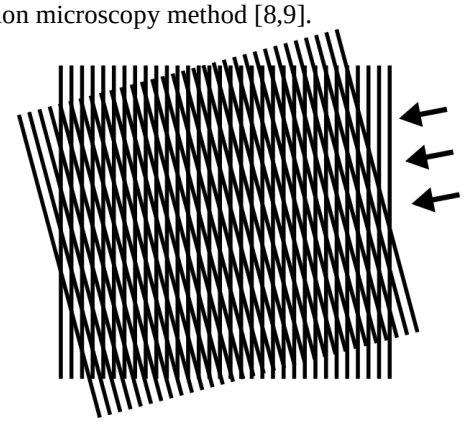
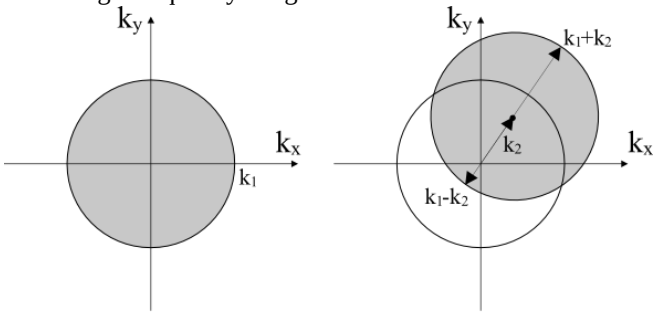


Fig. 1: Schematic image of the moiré effect. Appearance of a low-frequency fringe indicated by arrows by superposition of two high-frequency fringes

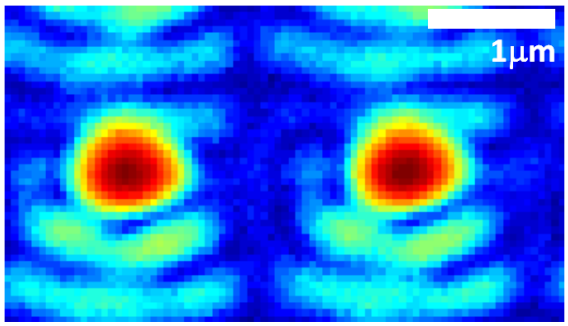


(a) (b)

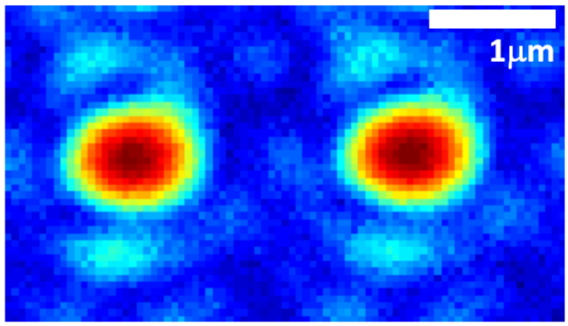
Fig. 2: Conceptual diagram of achieving high resolution by using structured illumination: (a) pass band of microscope in a spatial-frequency coordinate system, (b) shifted pass band due to structured illumination

3. LOW-SPECKLE IMAGING BY LOW-COHERENCE LIGHT

We used a laser (DPSS, wavelength 532 nm, spectral width 0.1 nm) and an SLD (center wavelength 669 nm, spectral width 7 nm) for structured illumination generated by two-beam interference. We compared the imaging of both methods with that of a conventional optical microscope image of a silicon substrate with 0.2-μm width dots. Figure 3 shows an example highlighting the speckle noise which depends on sample pattern. The speckle noise appearing as concentric circles corresponding to the center of each dot is mitigated through illumination by SLD. Moreover, the noise intensity using a laser is estimated over 10%, which can cause artefacts during image reconstruction. In general, it is believed that noise less than approximately 10% can be removed during reconstruction by using multiple-image stacking [6].



(a)



(b)

Fig. 3: Pattern-dependent speckle noise comparison of an optical microscope image under illumination by (a) laser and (b) SLD

4. EXPERIMENTAL SETUP

The structured illumination on the sample is generated by the interference of the outputs of a beam splitter. In this case, the theoretical value of the coherence length is given in following equation.

$$\text{Coherence length} = \frac{\lambda^2}{2\Delta\lambda} \quad (1)$$

Here, λ is wave-length and $\Delta\lambda$ is spectral width. From equation (1), coherence length of the SLD light source is 32 μm. We adopt a Michelson-interferometer system to realize the stringent demand for adjustment of the difference in

optical path length needed to achieve and precisely control the two-beam interference pattern. Figure 4 shows a schematic of the high-resolution optical microscope system. The structured illumination is generated in the Y-axis by two-beam interference and can be shifted in the Y direction by controlling a mirror on one optical path with a modulator. It allows that samples are not required to be manipulated. In order to maximize resolution and to improve contrast, we need to homogenize laser powers in both of optical paths. Therefore a second beam splitter is inserted to discard redundant laser power.

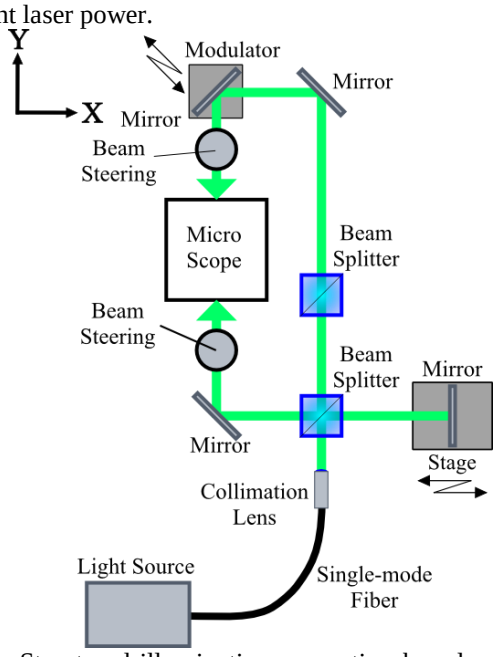


Fig. 4: Structured-illumination generation based on two-beam interference and an optical system for achieving high-resolution optical microscopy

5. CONVENTIONAL STRUCTURED ILLUMINATION

We performed an experiment for generating structured illumination by using the constructed optical system. We used a laser for generating the structured illumination in order to attain two-beam interference as a basic experiment. We observed a silicon substrate with 0.2-μm square dots (see Figure 5, made by NTT Advanced Technology Corporation). Because lasers interfere readily, structured illumination is generated over the entire field of view. The moiré effect can be seen between the structured illumination and the sample.

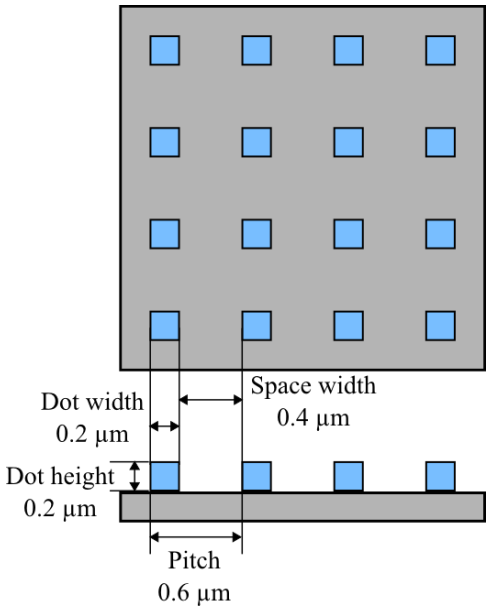


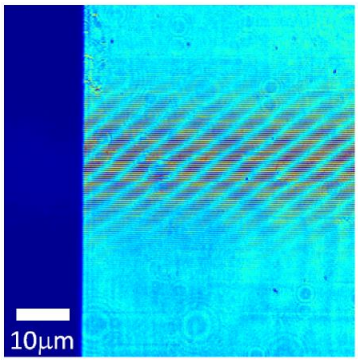
Fig. 5: Detail of a silicon-dot-patterned substrate for observation using structured illumination

6. EXPERIMENT FOR GENERATION OF LOW-COHERENCE INTERFERENCE STRUCTURED ILLUMINATION

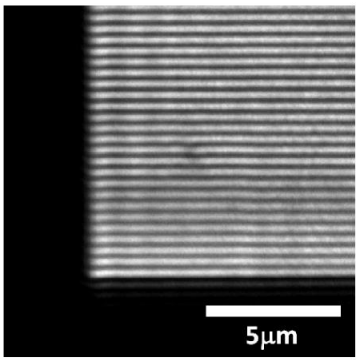
In an analogous manner, we performed an SLD experiment using the same silicon dot-patterned substrate used for the dark-field optical-microscopy imaging (with objective lens NA: 0.55). Figure 6 shows the observation. We confirmed the generation of a structured illumination with 0.47-μm pitches by analyzing the images obtained by shifting the sample stage in the Y-direction. Here, the theoretical value of pitch is given by following equation.

$$\text{Pitch} = \frac{\lambda}{2\sin\theta} \qquad (2)$$

From equation (2), we expect a pitch of 0.473 μm for two-beam interference of light incident at 45° with wavelength 669 nm. Shown in Figure 6 (a) is a color image of the entire field of view. The moiré fringes in the center part are generated by interference between the structured illumination and the sample pattern. In addition, Figure 7 shows a line profile of the Y-direction over the entire field of view. It can be seen that the contrast is maximized at a certain point (Figure 7 (a)) corresponding to zero path-length difference, which is a feature of low-coherence interference. As can be seen from the line profile, the structured-illumination area is approximately 30 μm, and it corresponds well to 32 μm, which is the theoretical value of the coherent length of the SLD.

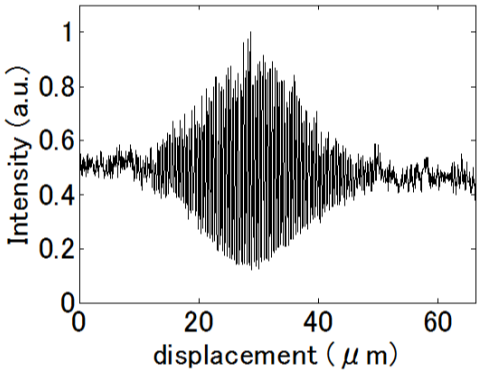


(a)

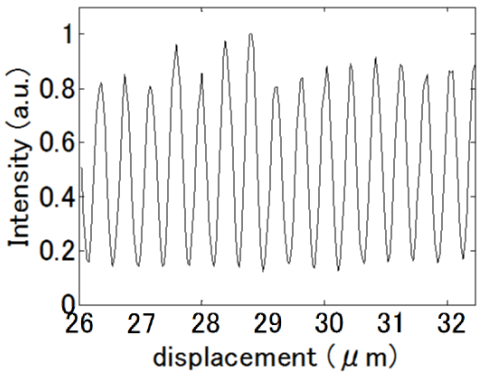


(b)

Fig. 6: Structured illumination generated by two-beam interference of an SLD: (a) entire of field of view, (b) close up



(a)



(b)

Fig. 7: Line profile of generated structured illumination: (a) entire field of view in Y-direction, (b) close up

7. EXPERIMENT FOR VALIDATION OF HIGH-RESOLUTION IMAGING

Here, we describe an experiment for validation of high-resolution imaging by using a silicon dot-patterned substrate for a resolution measurement (see Figure 8, made by NTT Advanced Technology Corporation). The experimental conditions are listed in Table 1. The observed sample has two types of pitches: 2.2 μm (in the X- and Y-directions) and 0.4 μm (in the Y-direction). However, because the diffraction limit is 0.5 μm, even when using an LED light source [10], a conventional optical microscope cannot resolve 0.4-μm pitch (see Figure 9). In this study, we use three images that are phase shifted in the Y-direction through structured illumination from two-beam interference of the SLD source; we seek to obtain high-frequency information through the image-reconstruction process. Figure 10 shows the reconstruction process represented in spatial frequency domain. Three images are extracted from an obtained image stacking and transformed to spatial frequency space (see Figure 10 (a)-(c)). As the result of mathematical computation, three images provide three solutions which correspond to origin, k_{y+}, and k_{y-} respectively (see Figure 10 (d)-(f) and Figure 11). Finally, reconstruction is performed by summing up these images with appropriate weights and inverse transformation to spatial domain. Figure 12 shows a successful result in reconstructing the high-resolution image. Although intensity unevenness is apparent in the Y-direction of the resulting image, the 0.4-μm pitch, which is smaller than the diffraction limit, is surely resolved because of the spatial-resolution improvement.

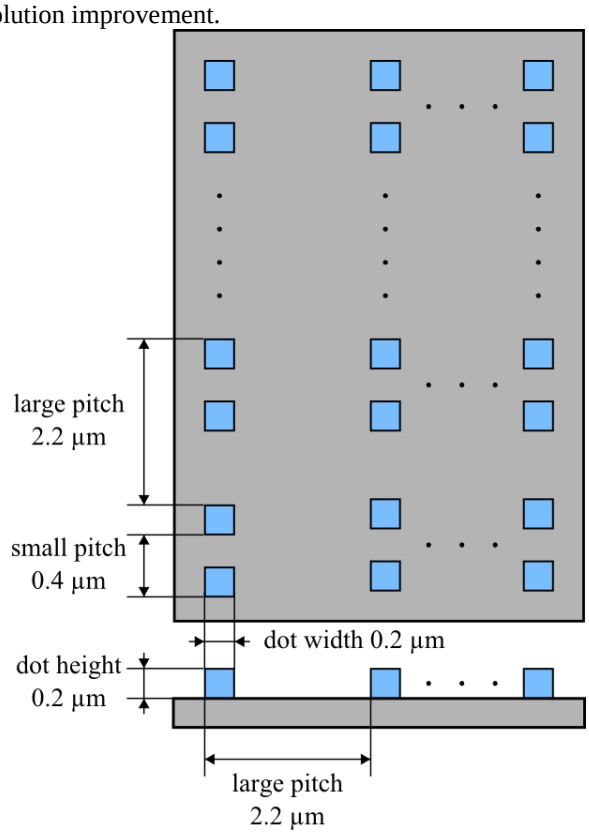


Fig. 8: Detail of silicon dot-patterned substrate for resolution measurement

Table 1: Experiment condition

SLD as light source wave length λ	0.669μm
Structured illumination pitch P	0.473μm
Observation magnification M	100
Numerical aperture of objective lens NA	0.55
Diffraction limit under the LED $DL1$	0.50μm
Diffraction limit under the SLD $DL2$	0.61μm

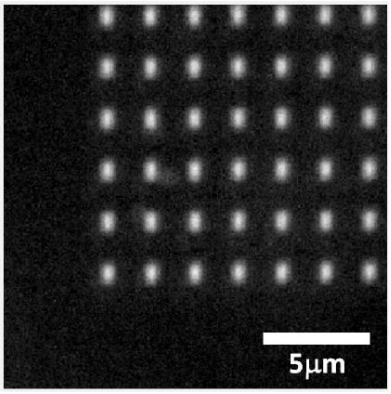


Fig. 9: Observation using conventional, white-light microscopy

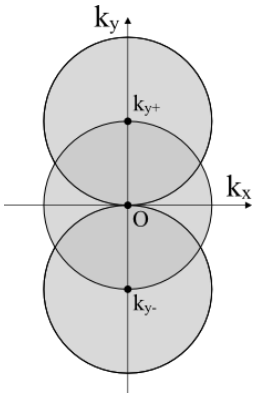


Fig. 11: Conceptual diagram of improved resolution in a spatial frequency domain

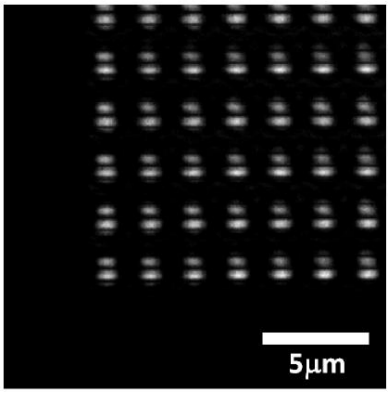


Fig. 12: Verification result for high-resolution imaging by structured illumination

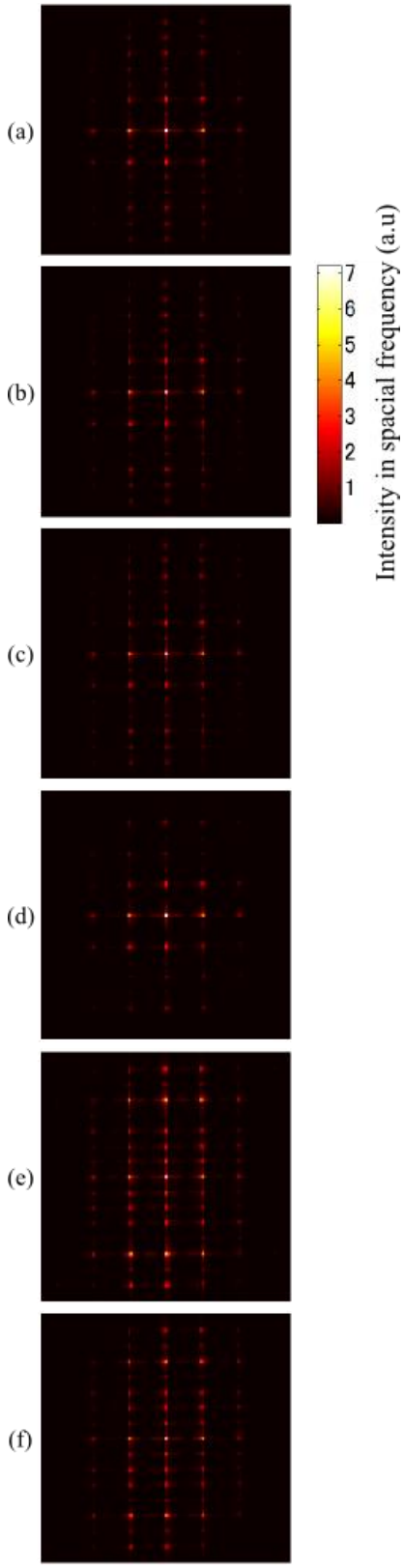


Fig. 10: Intensity distribution in spatial frequency domain (see figure 11) of the resulting images in the experiments, (a)-(c) spatial frequency distributions of phase shifted three images with the structured illumination, (d) reconstructed original spatial frequency distribution corresponding to an image with the uniform illumination, (e)-(f) reconstructed spatial frequency distributions with high spatial frequency components obtained by the moiré effect

8. CONCLUSION

In order to achieve high-resolution optical-microscope imaging for use in industrial fields, we suggested a method for generating structured illumination using two-beam interference of a low-coherence light source. We achieved high-resolution imaging with low speckle noise and verified its effectiveness through experiments. In future work, we seek to determine the phase shift value from the reconstruction process without any information about the positions of the mirror stages. We shall also consider applications for pattern height detection and highly precise control of illumination by using absolute optical-path length difference measurement, which is a feature of low-coherence interference.

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