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Coherent structured illumination microscopy using hexagonal-lattice illumination fields for enhancing the isotropy of 2D super-resolution

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Abstract
We present a method to improve the isotropy of 2D super-resolution in the coherent structured illumination microscopy (SIM) that can be implemented for imaging non-fluorescent samples. To alleviate the problem of anisotropic lateral resolution in the coherent SIM which previously employed the two orthogonal standing-wave illumination fields (referred to as the orthogonal SIM), we make use of a hexagonal-lattice illumination comprising three standing-wave fields that are simultaneously superimposed at the orientations equally divided in the lateral plane. A theoretical formulation is derived to rigorously model the coherent image formation with such simultaneous multiple-beam illuminations and a Fourier-domain framework is established to reconstruct an image with enhanced spatial resolution. Using a computer-synthesized resolution target to serve as a 2D coherent sample, we carried out numerical simulations to examine the imaging characteristics of our three-angle SIM compared with the orthogonal SIM. The investigation on the 2D resolving power for the various test patterns of different periods and orientations reveal that the orientation-dependent undulation of lateral resolution can be reduced from 27% to 8% in our three-angle SIM while the best resolution (0.54 times the resolution limit of conventional coherent imaging) in the directions of structured illumination is slightly deteriorated by 4.6% from that of the orthogonal SIM.

Keywords: Super-resolution microscopy, Coherent imaging, Image formation theory, Image reconstruction techniques.

1. Introduction

Diffraction of light has long been imposing a fundamental limitation on the spatial resolution attainable with conventional optical microscopy [1]. This has prompted challenging attempts to surpass the optical diffraction limit, leading to a number of super-resolution techniques such as STED, PALM, STORM, SIM, etc. with unprecedented far-field resolutions down to tens of nanometers [2]. Among them, structured illumination microscopy (SIM) [3] is unique in its principle to provide a way to improve resolution without relying on fluorophores. Nevertheless, it was not until recently that a substantial extension to the conventional SIM was made in 2012 by *Chowdhury et al.* [4] to render non-fluorescent, coherently scattering objects better resolvable in two dimensions (2D). They used illumination patterns built with two standing waves at 0° and 90° orientations, which undesirably resulted in "anisotropic super-resolution" as the extension of the effective detection band is smaller in other directions, particularly for 45° and 135° (see Fig. 1 (a)).

To improve the isotropy of lateral resolution in the 2D coherent SIM, we here present a modified framework employing a hexagonal-lattice illumination built with three standing-wave fields, instead of two orthogonal components considered previously [4], which allows the 2D image's frequency space to be filled more isotropically (see Fig. 1(b)). Based on a theoretical model derived for 2D coherent image formation under such multiple-beam illuminations, we establish an explicit framework on how to demodulate and process the mixed high-resolution information for a super-resolution-image reconstruction.

The validity of our proposed method is numerically investigated, demonstrating its improved performance in the 2D super-resolution compared to that of the 2-angle coherent SIM.

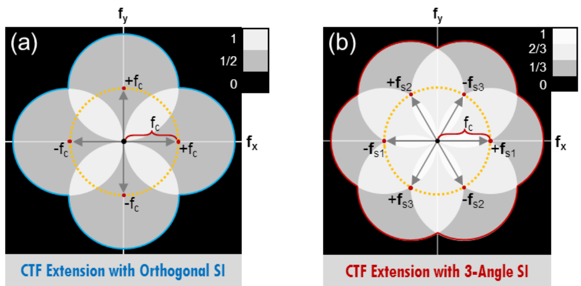

Figure 1. Extended CTFs with structured illuminations incorporating (a) 2 orthogonal standing-wave components (in the previous study) and (b) 3-angle standing-wave fields (in the present method), respectively.

2. Principle of coherent SIM with isotropic 2D resolution

The hallmark of the 3-angle coherent SIM is the hexagonal lattice-patterned illumination incorporating three standing-wave fields oriented at 0°, 120°, and 240° that are simultaneously superimposed in the sample plane as illustrated in Fig. 2(a). Using such an illumination in a coherent imaging is equivalent to a hypothetic extension of 2D coherent transfer function (CTF) as depicted in Fig. 1(b), provided that the high-frequency information multiplexed into a classical passband (with frequency cutoff at f_c) can be demodulated and shifted back to its original location.

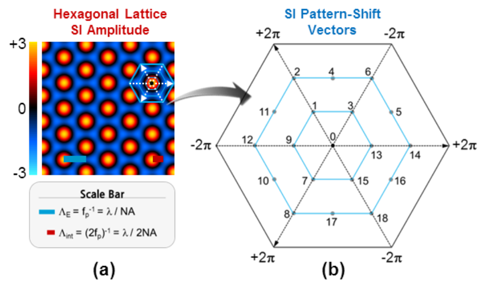

Figure 2. Amplitude distribution of the structured illumination (SI) field consisting of three-angle standing-wave components (a) and the 2D phase vectors by which the SI pattern is required to be translated laterally in the acquisition of a series of raw SIM image data (b). The 3-angle SI fields can be implemented experimentally by using a 2D programmable spatial light modulator (SLM) where a collimated laser beam is diffracted into multiple orders which are then recombined with appropriate demagnification to interfere at the microscopic sample.

We first theoretically modelled a coherent image formation under the 3-angle SI with a set of 19 translation vectors (as shown in Fig. 2(b)). Carrying out a rigorous comparison with that of a hypothetic super-resolution system specified by the 2D CTF in Fig. 1(b), we worked out a mathematical framework on how to demodulate and process the mixed high-frequency information to reconstruct a super-resolution image with a 2D-extended passband exceeding the diffraction limit δ_{DL} (= 1/ $2f_c$ = λ/2NA), from the raw SI image spectra containing a number of cross-correlation terms between the multiplexed sample spectra having different 2D frequency shifts [5].

3. Numerical Simulation

In order to investigate the validity of our 3-angle SIM and evaluate its 2D resolution performance, we carried out numerical simulations to produce a set of raw coherent SIM image data and reconstruct a resolution-enhanced image. A 2D resolution test target (1024×1024 pixels) was computer-synthesized with various test patterns (including groups of bar patterns, isolated single lines at different orientations, and a sector star pattern) in Fig. 3(a), where the radial bars of the sector star pattern have a cycle period equal to the diffraction limit of resolution δ_{DL} on the line delineated by the red circle.

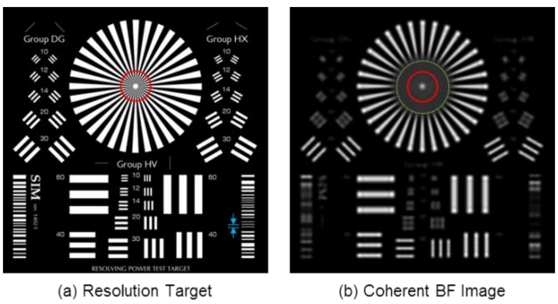

Figure 3. Computer-synthesized resolution test target (a) and its coherent bright-field (BF) image (b).

Using the coherent bright-field (BF) image in Fig. 3(b) as a reference (with an isotropic resolution of δ_{CBF} = 1.67×δ_{DL} according to the Rayleigh's criterion), the reconstructed images from the two schemes of coherent SIM under consideration were compared in Fig. 4(a) and 4(b). Resolution improvements in both coherent SIMs were found to be obvious that the same sector star patterns appeared more sharply and rendered the features closer to the center (inside the green circles indicating the actual limit of coherent BF resolution) clearly visible. In the direction around the SI orientations, the orthogonal SIM allowed its best resolution of $\delta_{SI\text{-}2B}$ = 0.87×δ_{DL} (at 0° and 90°) and the 3-angle SIM showed a super-resolution of $\delta_{SI\text{-}3B}$ = 0.91×δ_{DL} (at 0°, 60°, and 120°), nearly a half of the coherent BF resolution. In the direction midway between the SI orientations, however, some degrees of resolution degradation were apparent from the bar-pattern groups at different orientations. It was found that our 3-angle SIM leads to less degraded resolution of $\delta_{SI\text{-}3W}$ = 0.98×δ_{DL} (at 30°, 90°, and 150°) than the worst resolution $\delta_{SI\text{-}2W}$ = 1.11×δ_{DL} (at 45° and 135°) of the orthogonal SIM, which is also evident from Figs. 4(c) and 4(d), displaying the footprints of the corresponding Fourier spectra for the two coherent SIM images. Interestingly, our 3-angle SIM was also found to significantly alleviate the "image intensity" undulation depending on the pattern orientation, being predominant in the orthogonal SIM.

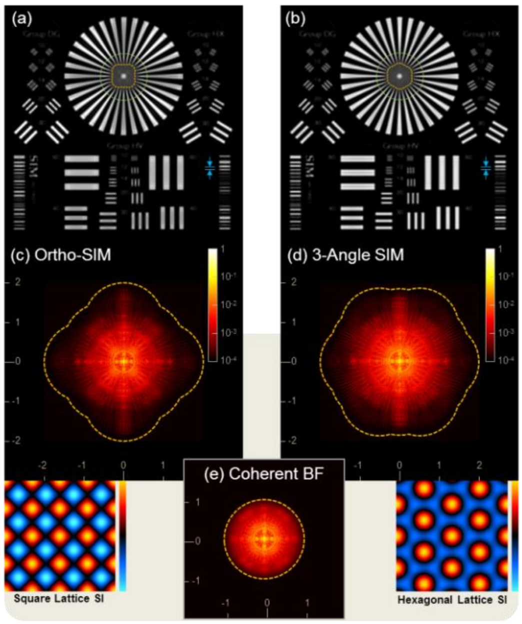

Figure 4. Reconstructed images of (a) the orthogonal SIM and (b) the 3-angle SIM obtained for the resolution test target in Fig. 3(a). Below the images are the corresponding spatial-frequency spectra for (c) the orthogonal SIM and (d) the 3-angle SIM, compared with (e) that of the conventional coherent BF image shown in Fig. 3(b).

4. Conclusion

We have proposed a 3-angle coherent SIM that exploits a hexagonal-lattice illumination consisting of three simultaneous standing-wave fields. Detailed numerical investigation has been carried out to verify its feasibility and evaluate the 2D resolution performance compared with that of the existing coherent SIM. Our 3-angle SIM has been confirmed to be capable of detecting the sample's high-resolution information down to 0.54 times the coherent diffraction limit δ_{CBF} and reducing the orientation-dependent degradation in 2D super-resolution from 27.6% to 7.7% (by a factor of about 3.6).

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