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Four-Frame Structured Illumination Microscopy Based on Checkerboard Pattern

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INTRODUCTION & AIM

Structured illumination microscopy (SIM) is a mainstream super-resolution imaging technique, effectively doubling the lateral resolution. Traditional 2D-SIM requires nine raw images to reconstruct a single super-resolution image. Reducing the exposure time can mitigate motion artifacts but at the cost of image quality; conversely, increasing the exposure time can heighten phototoxicity, impacting the overall observation time and imaging efficiency.

To address these challenges, we propose a novel method called 4 frame checkerboard SIM (4CSIM), which replaces sinusoidal illumination with a checkerboard-patterned structure, requiring only four raw images to achieve super-resolution imaging. 4CSIM enhances both the acquisition frame rate and reconstruction speed of SIM technology, making real-time video imaging feasible.

METHOD

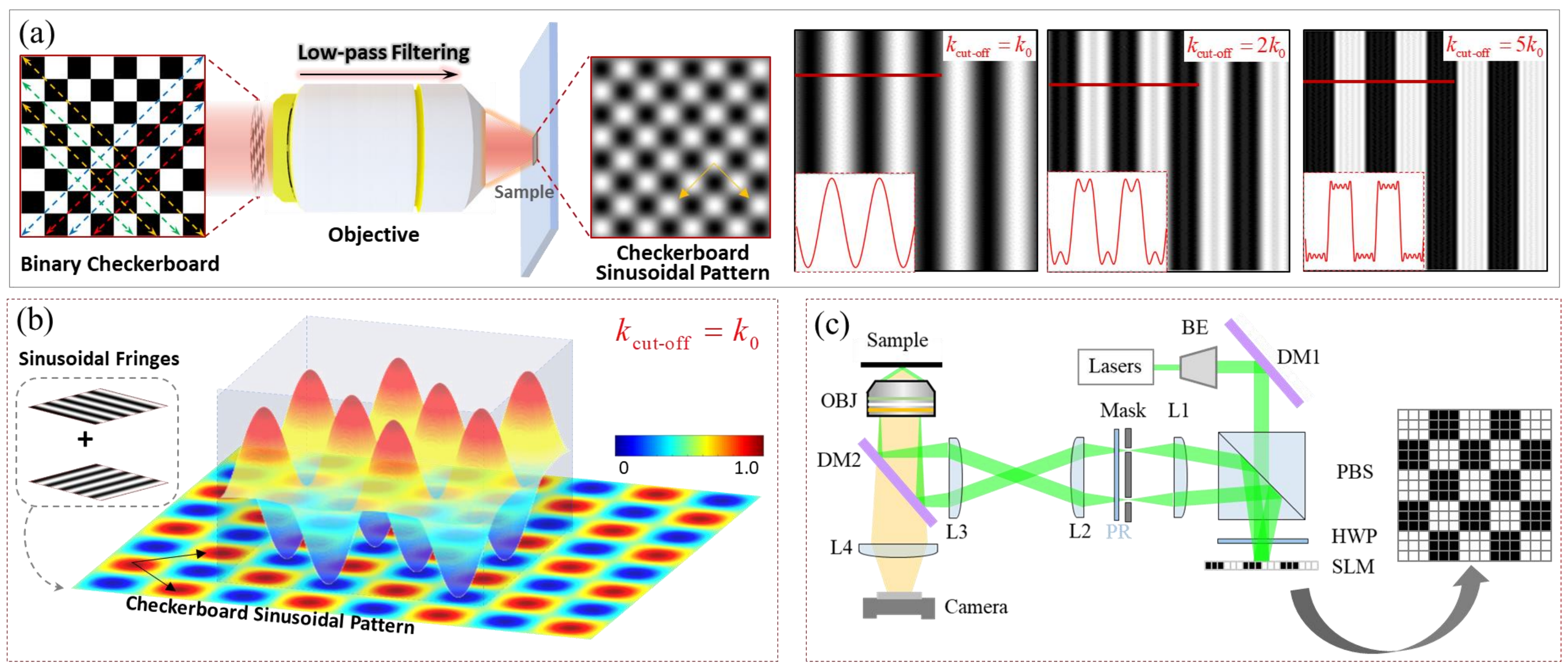

Fig.1. Principle of checkerboard structured illumination. (a) Binary pattern filtering achieves checkerboard sinusoidal illumination. (b) Intensity distribution of checkerboard illumination. (c) A 4CSIM experimental setup based on a spatial light modulator (SLM).

The checkerboard pattern used in 4CSIM is shown in Fig. 1. The first generation method directly employs digitally encoded binary patterns, which are filtered through the objective lens (Fig. 1(a)). The distribution of the checkerboard structured illumination is shown in Fig. 1(b), equivalent to the superposition of orthogonal sinusoidal fringes. Another method for generating the pattern is to encode the SLM as a two-dimensional grating to produce the desired pattern (Fig. 1(c)).

Checkerboard illumination expands the aliased spectral components from three to five in classical methods. The 4CSIM performs a discrete analysis of the spectrum, enabling point-by-point spectral reconstruction on a pixel basis, as illustrated in Fig. 2. In the frequency domain of the raw SIM image, each pixel within the cutoff frequency is the superposition of five components at corresponding positions. By considering wide-field information, sufficient reconstruction can be achieved with just four images.

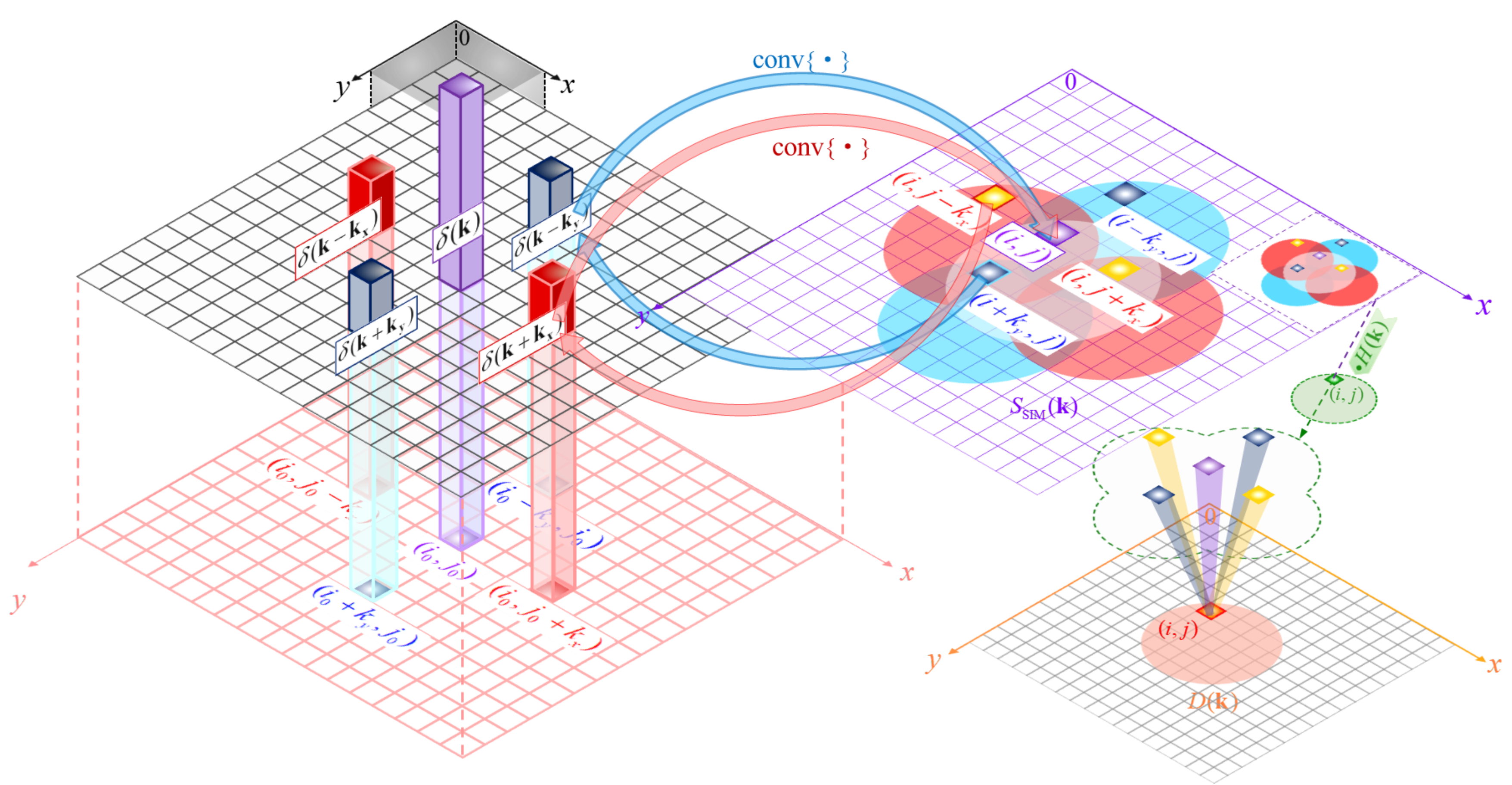

Fig.2. Schematic diagram of the discrete model for generating raw SIM images based on the proposed method.

RESULTS & DISCUSSION

Figures 3 and 4 provide simulation and experimental validation of the proposed method. Compared to wide-field imaging, our results demonstrate super-resolution effects. When compared to the classical SIM method, there is an unavoidable slight decrease in imaging quality; however, the acquisition speed has improved by more than two times. In comparison with other existing frame-reduction methods, 4CSIM achieves substantial gains in reconstruction speed by saving computational resources through point-by-point reconstruction. Other methods sacrifice reconstruction time due to iterative calculations or large matrix operations, making it difficult to meet the demands for real-time reconstruction.

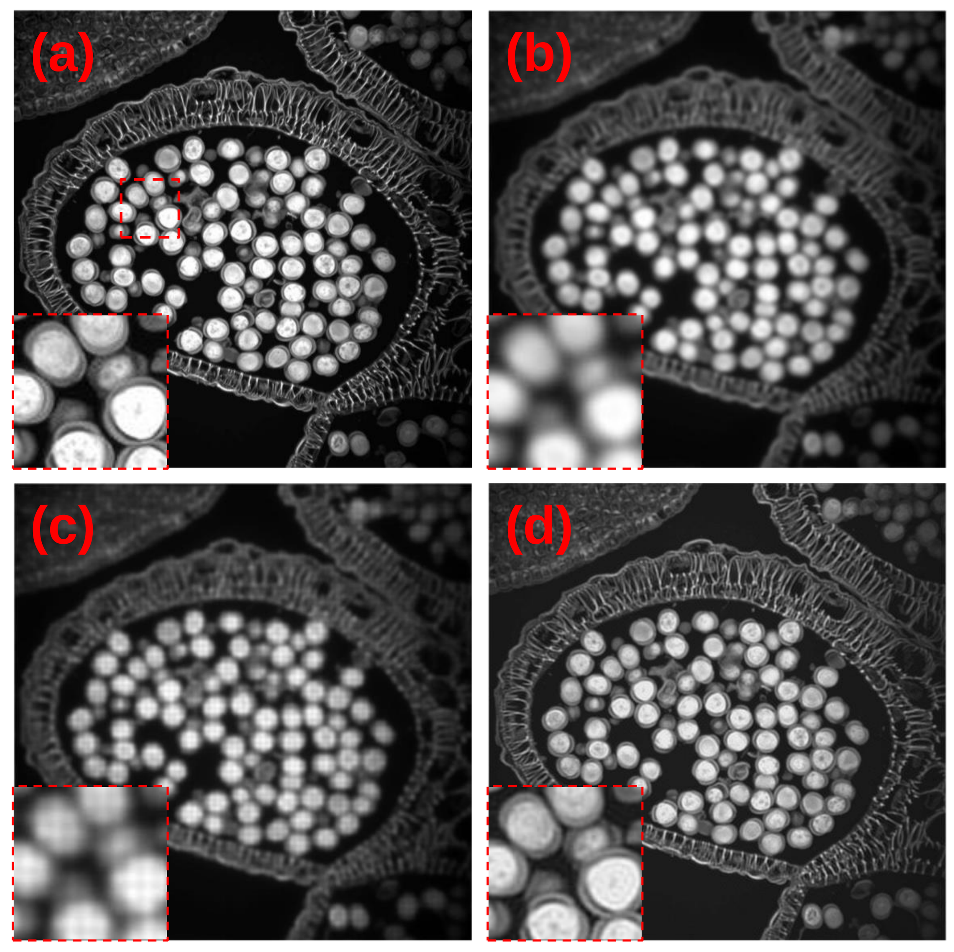

Fig.3. 4CSIM Simulation Verification. (a) Ground truth. (b) Wide-field image. (c) Raw image under checkerboard structured illumination. (d) Super-resolution reconstruction result.

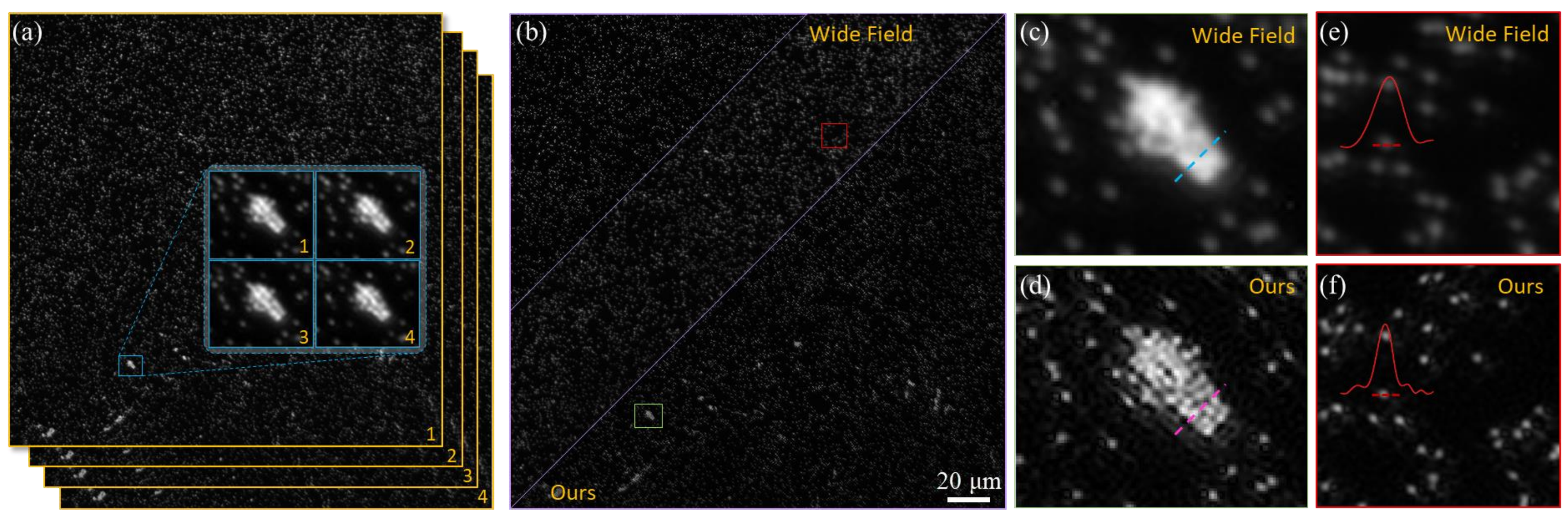

Fig.4. (a) Four original images. (b) Comparison between the wide-field image and the reconstructed super-resolution image. (c-f) Comparison of localized enlarged details.

CONCLUSION

In conclusion, the checkerboard illumination pattern enables this "point-wise reconstruction" method, with the reconstruction process involving only three-dimensional matrix operations. 4CSIM significantly accelerates image reconstruction due to its non-iterative nature and low memory usage, avoiding the trade-off between a high frame rate acquisition and reconstruction speed observed in 4SIM methods.

The proposed method achieves 2x super-resolution reconstruction using only four images while significantly reducing reconstruction time to levels comparable to or even lower than those of the classical SIM method, thereby establishing a solid foundation for dynamic real-time super-resolution cellular observation.

FUTURE WORK / REFERENCES

In the future, we will focus on developing a three-frame SIM reconstruction algorithm using methods not related to deep learning.

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