Hybrid Illumination Angle Calibration for Fourier Ptychography Microscope

Chen Liang,[1] Fan Feng,[2] Pingyong Xu,[3,4] and Heng Mao[1,*]

[1] *LMAM, School of Mathematical Sciences, Peking University, Beijing 100871, China*
[2] *GBA Institute of Collaborative Innovation, Guangzhou 510300, China*
[3] *Key Laboratory of RNA Biology, Institute of Biophysics, Chinese Academy of Sciences, Beijing, 100101, China*
[4] *National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China*

[*] *heng.mao@pku.edu.cn*

Abstract: We proposed a hybrid calibration method for quantitative correction of illumination angle misalignments in Fourier ptychography microscope. This method combines system parameter pre-calibration with online sample-include calibration to ensure high-quality results.

1. Introduction

Fourier ptychography microscopy (FPM) is a computational imaging technology known for its high resolution and high throughput, initially introduced by Guoan Zheng in 2013 [1]. The reconstruction process in FPM relies heavily on an accurate imaging model. Misalignment of the illumination angle can result in significant artifacts in the reconstructed images, making reliable and precise calibration methods for the illumination angle crucial. Various calibration techniques have been proposed to address the issue of illumination angle misalignment in FPM. In 2016, Jiasong Sun and colleagues [2] proposed an iterative method to correct global position misalignment using simulated annealing and non-linear regression. This approach is both efficient and robust against noise, but it requires the LEDs to be arranged in a regular pattern and pre-aligned to their desired positions. If the illumination angle is significantly misplaced, the method can become time-consuming. In 2018, Regina Eckert and colleagues [3] proposed a two-part self-calibration method. This method first recovers brightfield illumination angles by analyzing individual frequency regions, and then refines both brightfield and darkfield angles through spectral correlation. This method demonstrates robustness across various FPM setups with different source types. However, it requires a large numerical aperture (NA) objective (NA > 0.2) to accurately determine LED positions from a sufficient number of brightfield images. For the calibration of darkfield images, this method is dependent on the initial value.

Our previous work [4] proposed a sample-independent, phantom-based geometric calibration method to correct illumination angle misalignment in FPM. To adapt to illumination angle disturbances caused by scattered samples in vibrating environments, we combine our previous calibration method with a sample-include calibration method. In this approach, the phantom-based calibration provides the initial value for subsequent online alignment processes. We compared this hybrid method with existing calibration methods and demonstrated its effectiveness across various samples, showing superior performance.

2. Proposed hybrid illumination angle calibration method

In FPM, illumination angle misalignment can be categorized into two primary types. For thin samples, the illumination angle is predominantly determined by system parameters. In FPM, the spatial frequency of an LED at a given position $\mathbf{x}_i = (x_i, y_i, z_i)$ can be obtained as: $\mathbf{k}_i = \frac{(x_i, y_i, z_i)}{\sqrt{x_i^2+y_i^2+z_i^2}} k_0$, where $k_0 = \frac{2\pi}{\lambda}$ is the wave vector in vacuum. To determine the wave vector $\mathbf{k}_i$, the LED's position $\mathbf{x}_i$ must be calibrated. Our previously proposed phantom-based calibration method can calculate the geometric intersection of multiple rays to calibrate the LED's position and, subsequently, the illumination angle. For thick, scattering samples, the illumination angle is primarily influenced by system parameters but is also affected by sample scattering. In this case, sample scattering introduces small perturbations around the system-determined illumination angle. Therefore, we propose a hybrid calibration process: first, employ the phantom-based calibration method to calibrate the system parameters. Second, further correct the illumination angle misalignment caused by light deflection from different samples based on the obtained system illumination angles. It is evident that for existing sample-dependent methods, utilizing the system illumination angle calibration results as the initial values for algorithm iteration is a straightforward and effective strategy. In the following experiments, the phantom-based calibration method provides initial values for an online calibration method. We select self-calibration [3] as the example of the online calibration method to illustrate our proposed divide-and-conquer calibration strategy.

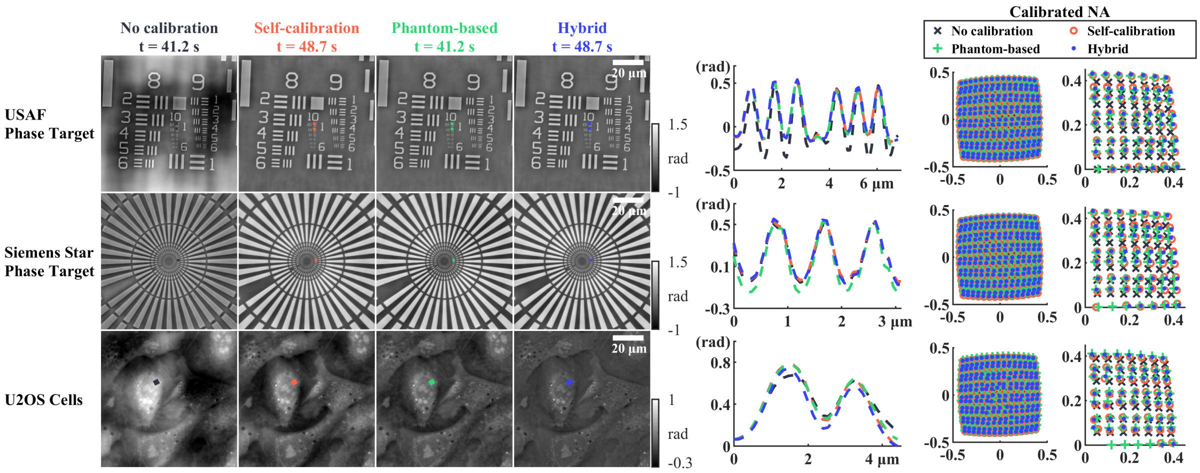

Fig. 1. The FPM phase reconstruction results of the USAF target, Siemens star phase target, and U2OS cells are evaluated under four different calibration methods: no calibration, self-calibration [3], phantom-based calibration, and hybrid calibration. The reconstruction time reported includes both calibration and reconstruction processes. A comparison of phase distributions corresponding to the colored lines is presented. Additionally, the calibrated NAs and their corresponding zoomed-in views are also presented.

We demonstrated our proposed method using a USAF phase target, a Siemens Star phase target (Benchmark Technologies, Inc.) and live U2OS cells, as shown in Fig. 1. All methods were evaluated using the same Ptychographical Iterative Engine (PIE) algorithm [5] for comparison. LEDs are assumed to be arranged in an orthogonal grid when no calibration. However, in reality, each LED bead is connected in series, with the rows daisy-chained end-to-end, resulting in staggered calibration results across rows. For the USAF and Siemens star phase targets, all three methods reconstruct similar quality reconstructions. However, the self-calibration method exhibits some artifacts in the background of the USAF phase target. Both the phantom-based method and the hybrid method produce comparable results on these samples, effectively correcting most illumination angle misalignments and achieving resolutions close to theoretical limits. Additionally, the NAs from these two methods are relatively close and follow similar patterns, indicating that sample scattering only causes minor deviations in the illumination angles. This also suggests that for samples with a uniform refractive index, such as targets, calibrating the system illumination parameters alone is sufficient. However, for live cell samples, the hybrid method outperforms the phantom-based method due to its consideration of illumination errors induced by the sample itself. In contrast, the self-calibration method suffers from more pronounced artifacts due to its initial value deviations.

3. Conclusions

To address the issues of illumination angle misalignment in FPM, we propose a hybrid calibration method that first calibrates the illumination parameters of the system, followed by correcting for illumination angle deviations induced by the sample itself. We have demonstrated the effectiveness of our proposed method across various samples.

References

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