

Purity-based self-calibration in ptychography

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Calibration of ptychographic setup properties, such as the sample-detector axial distance, is crucial for quantitative and high-resolution reconstructions. This study proposes a calibration scheme based on the degree of coherence (i.e., purity) of the probe in mixed-state ptychography. Compared to established sharpness metric-based methods, it is significantly more robust and applicable to arbitrary samples. A numerical study and validations with various experimental datasets have been conducted to verify the performance of the proposed method, including the application to high-contrast samples and weakly-scattering biological samples. This calibration scheme can significantly enhance the image quality, the precision of wavefront sensing, and the reliability of quantitative analysis for ptychography. Potentially, we believe this optimization method can find further applications in other areas of computational imaging.

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Introduction. Ptychography, as a scanning coherent diffraction imaging (CDI) method, has rapidly developed into an advanced imaging technology that facilitates high-resolution microscopy [1–3]. In a ptychographic measurement, an illumination beam (probe) is scanned over an extended sample, capturing a sequence of diffraction patterns, which allows for the reconstruction of the complex-valued object and illumination. This technique is of particular interest in short wavelength spectral regions like the extreme ultraviolet (EUV) and x-rays [4], where absorptive losses and resolution limitations inherent to inefficient imaging optics can be avoided [5], allowing for high-resolution imaging limited only by the wavelength and the numerical aperture (NA). Furthermore, EUV/x-ray radiation enables excellent material-specificity from various elemental absorption edges [6]. The structural and chemical information [7] can be extracted quantitatively through the reconstructed complex transmission of specimens.

However, special attention has to be paid in the calibration of the ptychographic setups to ensure accurate knowledge of

the experimental parameters involved in the reconstruction [8]. Even minor misalignments can lead to a model mismatch and result in severe errors in the high-resolution image. To this end, various self-calibration techniques have been demonstrated to enhance the experimental robustness of ptychography, addressing issues such as lateral [9,10] and axial [11–15] position misalignment, probe instability [16], partial coherence [17], and sample tilt in reflection-mode ptychography [18]. Another promising approach involves the use of automatic differentiation and machine learning toolboxes [19], which have shown the ability to enhance both reconstruction speed and image quality [20]. Particularly, the miscalibration of the axial distance between the sample and detector not only leads to degraded reconstructions with reduced resolution and visible artifacts but also results in incorrect reconstruction of the quantitative phase and amplitude, thus reducing the reliability of the image's interpretation. Previous autofocusing methods (i.e., zPIE [13]) based on sharpness edge metrics encounter difficulties when specimens lack clear sharp edges, a common issue in biological imaging. Here, we propose a general calibration method based on the purity of the reconstructed probe, previously introduced in spatial coherence theory [21,22]. Aside from quantifying the overall coherence of the imaging system, we demonstrate here that the purity can serve as a metric for calibrating the axial distance. This approach is broadly applicable to various sample types and does not rely on a sharpness metric [12–15].

Purity in a ptychographic reconstruction. Based on partial coherence theory, the decoherence effect in a ptychographic setup is influenced by factors like source spatial and temporal incoherence, high-frequency sample vibrations [23], the detector point spread, and background noise. Decoherence effects can be accounted by mixed-state ptychography [17]. The measured far-field intensity $I_j(q)$ at position j is described by an incoherent sum over the product of multiple probe modes $P_m(r)$ and the object $O(r)$ propagated to detector plane as follows:

$$I_j(q) = \sum_m |\mathcal{F}[P_m(r) \cdot O(r - r_j)]|^2. \quad (1)$$

Here $\mathcal{F}[\cdot]$ denotes the Fourier transform operator modeling the far-field diffraction.

The proposed method is based on the reconstructed coherence metric (i.e., purity). To obtain this metric, several iterations of

momentum-accelerated Ptychographic Iterative Engine (mPIE) [24] through all positions j with an initial guess of axial distance z_{in} are performed to retrieve the wavefront of the object O and probe P_m . The mutual intensity [25] is then calculated from the reconstructed coherent modes of the probe as follows:

$$J(r_1, r_2) = \sum_m P_m^*(r_1) P_m(r_2), \quad (2)$$

which can be decomposed into $J(r_1, r_2) = \sum_m \lambda_m \phi_m^*(r_1) \phi_m(r_2)$.

Here λ_m represents the intensity contribution of the corresponding orthonormal modes ϕ_m , i.e., relative power content. The concept of purity is defined by normalized eigenvalues [21,22] as follows:

$$\mu = \sqrt{\sum_m \lambda_m^2 / \sum_m \lambda_m}. \quad (3)$$

Here $\mu \in [0, 1]$ demonstrates the concept of “purity” of the optical field, particularly in quantum optics [26]. The estimated intensity is propagated back to the real space during the reconstruction algorithm. If the propagation distance z_{in} is set incorrectly, there will be a mismatch in the diffraction integral connecting real and reciprocal space, resulting in a stretching deformation of the retrieved image for both object and probe [12]. This deformation and resolution degradation manifest as a reduction in purity and cannot be recovered through subsequent numerical propagation. Our work demonstrates that purity depends on axial distance when other experimental properties, such as illumination wavelength, are kept constant. Here mPIE engine from the Ptychographic framework Ptylab [27] is used to reconstruct at different z_{in} to refine the z -value direction toward a higher purity. The axial distance that leads to the highest reconstructed purity corresponds to the truth and yields the highest image quality.

Numerical verification. We conducted a numerical study on a synthetic transmission Ptychography dataset to examine the usability of this method. Here we simulate a binary Siemens star object illuminated by a monochromatic beam with a wavelength of 13.5 nm, which is highly relevant for our state-of-the-art high harmonic generation-based EUV source [28]. A 10 μm spiral mask is utilized to generate structural illumination [29]. The sample was scanned with a 20 μm Fermat-spiral scan grid (scan positions, 255; overlap, 87%; photon flux, 1.29×10^{11} photons). The object exit wave is numerically propagated to the far field (sample-detector distance, 32.5 mm; detector pixels, 1024×1024 ; pixel size, 11 μm ; noise statistics, a mixture of Poisson and Gaussian distribution). The ground truth structured illumination on the object is modeled as an incoherent sum of four spatial modes (shown in a). The dominating normalized eigenvalue λ_1 is calculated to be 92.54%, indicating a high coherence. The ground truth of purity μ_{tr} is then determined to be 92.45% at ground truth $z_{tr} = 32.5$ mm.

Next, independent reconstructions were performed with multiple axial distances. Fifty iterations of mPIE with four spatial modes are used for reconstruction at each z . Here, the concept of axial tolerance has to be introduced since it determines the axial step size of the purity scan. Unlike conventional microscopy, the axial tolerance in Ptychography is not directly linked to the concept of depth of field (DOF), which limits the thickness of the investigated object. Here, the criterion for the axial scan step size is determined by whether the axial perturbation causes a single-pixel distortion in any region of the reconstructed object

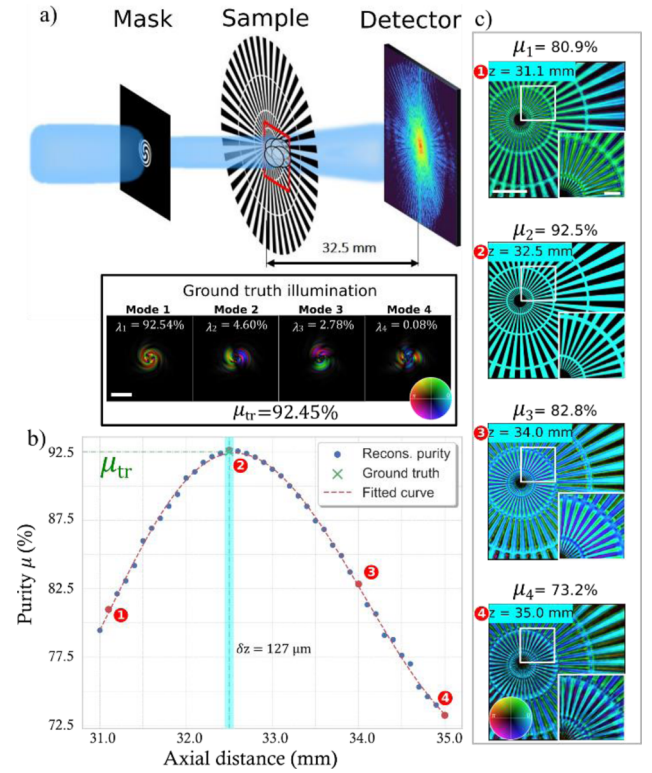


Fig. 1. Purity-based optimization on synthetic Ptychographic data. (a) Simulation of a transmission Ptychographic setup. Scale bar, 10 μm . (b) Reconstructed purity versus estimated axial distances. (c) Reconstructed complex transmission of the objects selected in (b). The brightness and hue encode modulus and phase. The scale bars correspond to 5 μm and 1 μm of the insets.

space. Incorporating the sampling condition of a pixel size Δx in Ptychography $\Delta x = \lambda z / D$, the axial tolerance δz can be derived as follows:

$$\delta z = \frac{\lambda z_{tr}^2}{rD - \lambda z_{tr}}, \quad (4)$$

where D is the detector size and r is the largest physical distance from the center to the edge of the scan grid. A detailed discussion of this model is provided in Supplement 1. Based on Eq. (4), the tolerance δz in this synthetic scenario is calculated to be 127 μm , whereas the DOF of this system is only 450 nm. This demonstrates that a typical high-NA Ptychographic setup in the EUV range has a tolerance of several hundred micrometers, with an axial distance of several tens of millimeters. This makes precise determination of the distance challenging, highlighting the significance of our approach.

Repeated scans with same input parameters are conducted to prove the reproducibility. The resulting purity is plotted against the axial distance over a range of 31.0 mm–35.0 mm in Fig. 1(b). For clarity, a fifth-order polynomial is included to identify a local maximum at 32.5 mm, where the purity reaches 92.5%, aligning with the ground truth. In Fig. 1(c), reconstructions are selected to illustrate the quality differences with the optimized reconstruction occurring at the ground truth z_{tr} with optimum purity μ_{tr} . Artifacts of the reconstructed object are evident at incorrect z_{in} as can be seen in Fig. 1(c), cases 1, 3, and 4. In contrast, case 2 demonstrates that the reconstruction at the ground truth $z_{tr} = 32.5$ mm provides optimal image quality, with the reconstructed purity closely matching the illumination purity. In this

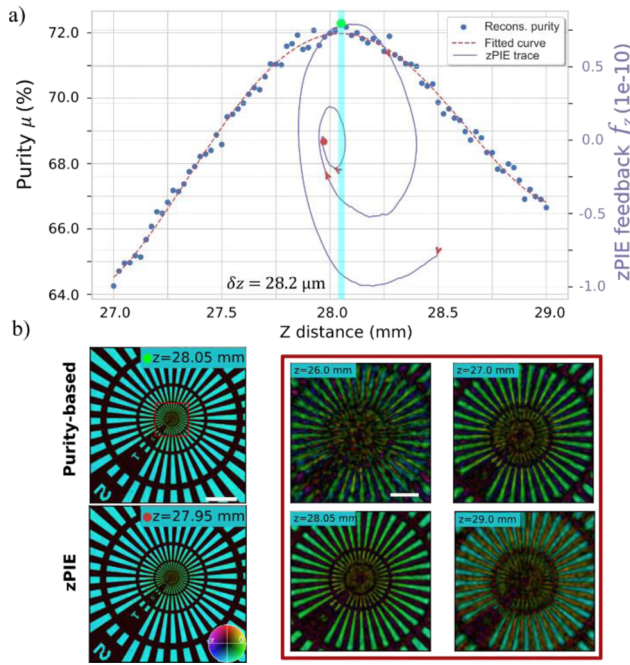


Fig. 2. Verification of experimental dataset: EUV image of Siemens star [30]. (a) Illustration of purity and zPIE trace (purple curve), with the sampling threshold indicated by the blue shading. (b) Complex object transmission at $z = 28.05 \text{ mm}$ (purity-based result, green dot) and $z = 27.95 \text{ mm}$ (zPIE result, red dot). Insets show the enlarged $10 \mu\text{m}$ center (red) of the reconstruction at three different axial distances, with the optimal at $z = 28.05 \text{ mm}$. Brightness and hue encode modulus and phase. Scale bar, $10 \mu\text{m}$ and $2 \mu\text{m}$ (inset).

scenario, selecting the axial distance based on maximum purity results in the best image quality. An additional numerical study using fully coherent illumination, included in Supplement 1, further illustrates the broad applicability of this method.

Experimental verification with a test binary sample. To assess the method's performance, we tested the technique on published EUV datasets and compared it with the established sharpness edge metric-based method zPIE [13]. A raw dataset recorded on a Siemens star with structured EUV illumination at 13.5 nm wavelength [30] was tested. The sample-detector distance of this dataset was reported as 28 mm . The reconstruction is conducted using mixed-state mPIE with the probe decomposed to four spatially incoherent modes. During the test, we observed that zPIE is highly sensitive to selective parameters, particularly the initial guess to converge within a reasonable number of iterations. In this case, an initial guess of 28.5 mm was chosen. Here, the feedback term f_z introduced in the original work [13] serves as a convergence indicator as shown in Fig. 2(a), plotted alongside the purity scan. Note that the tolerance δz is calculated to be $28.2 \mu\text{m}$ (blue shadow in Fig. 2(a)), while the DOF is only $1.79 \mu\text{m}$. Details of zPIE settings can be found in Supplement 1. In Fig. 2(a), f_z forms a spiral trajectory that converges to 27.95 mm and shows close alignment with the optimum purity at $z = 28.05 \text{ mm}$. Figure 2(b) shows the reconstruction at both $z = 28.05 \text{ mm}$ and $z = 27.95 \text{ mm}$, demonstrating equally good quality within $100 \mu\text{m}$ —early 100 times the DOF—thereby illustrating the concept of axial tolerance in ptychography. A detailed comparison is provided in Supplement 1.

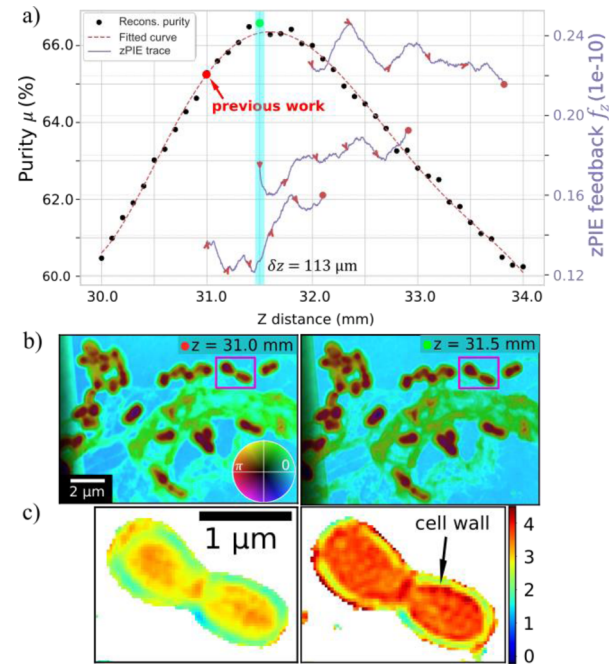


Fig. 3. Verification on biological sample of *Escherichia coli* dataset [30]. (a) Illustration of purity scan and zPIE feedback metric (purple curves). The optimum purity position is 31.5 mm , and the zPIE method fails to converge with various initial guesses and settings (here, three trajectories are illustrated for example). (b). m-s OPR-based reconstruction of previous work and optimum purity position. (c) Scattering quotient map of the selected cells under division for chemical component analysis.

As expected, reconstructions at incorrect distances result in poor image quality. In this case, we demonstrate that the purity-based calibration method exhibits consistency with zPIE for a binary sample with sharp edges. It also shows superior robustness to the initial guess compared to sharpness-based methods, such as zPIE.

Effect of z-miscalibration on quantitative analysis. Next, the impact of the axial distance on the quantitative analysis of the reconstruction, especially the compositional analysis on biological samples, is investigated. We applied the same procedure as described above on the dataset in Ref. [31]. Here, a dried and unstained microbial sample of *Escherichia coli* (*E. coli*) was prepared on a 50 nm SiN_x membrane and was measured at 13.5 nm wavelength. Information about the interior material composition was extracted from the reconstructed EUV image through an analysis of the scattering quotient [32].

For reliable quantitative image analysis, mixed-state orthogonal probe relaxation (m-s OPR) [16,33] is utilized, resulting in four mixed states with each four OPR modes. In the original publication, an axial distance of 31 mm was assumed. However, the purity analysis (Fig. 3(a)) indicates a purity-optimized distance of $z = 31.5 \text{ mm}$. For this dataset, zPIE fails to achieve consistent convergence (three trajectories in Fig. 3(a)), regardless of variations in initial guesses and setting parameters, presumably due to the absence of sufficient sharp features in the sample. The detailed zPIE parameters and discussion are provided in Supplement 1. Figure 3(b) displays the complex EUV images of the *E. coli* clusters reconstructed at the original and purity-optimized z position. The region where a cell is undergoing

division (purple window in Fig. 3(b)) was selected to calculate the scattering quotient, highlighting the distinction between the cell body and its boundary. Figure 3(c) illustrates the scattering quotient for the selected regions. It is observed that the boundary, identified as the cell wall, appears much sharper at the purity-optimized position, and the overall scattering quotient is higher than the original reconstruction in Fig. 3(b)). According to Table 1 in the original publication [31], the dominance of carbohydrates ($f_g = 3.5$) in the cell boundary should give a scattering quotient in the range of 3.5. The center of the cell bodies, which predominantly contains proteins (4.3), DNA (3.6), and lipids (6.0), should exhibit an averaged scattering quotient value of approximately 4. Apparently, the original scattering map (Fig. 3(b)) was inaccurate, as it shows an overall scattering quotient among the cell bodies averaging approximately 3.02 ± 0.12 . In contrast, the reconstruction with the optimum purity (Fig. 3(c)) provides an averaging scattering quotient of 3.77 ± 0.09 , offering a more accurate and physically plausible chemical mapping.

Discussion. In this paper, we demonstrate that the reconstructed purity serves as a straightforward and effective criterion for ptychographic calibration like sample-detector distance. This criterion reflects the coherence of a diffractive imaging system. Any degradation for the given datasets indicates a mismatch, caused by experimental miscalibration, between the forward model and the measured intensity in reciprocal space. We perform a numerical study to verify the dependency of purity on the input estimation of axial distance. The experimental validation on two EUV datasets, Siemens star and *E. coli* bacteria, demonstrates the method's feasibility. The axial calibration accuracy is confirmed by the sharpness metric-based method zPIE and also demonstrates its superior applicability and robustness across a wider range of sample conditions. Furthermore, the correction in chemical components analysis of the biological sample indicates the importance of accurate axial distance calibration to extract quantitative information.

The key benefit of purity-based optimization is that it is not reliant on sharpness metrics, making it particularly suitable for biological samples, which often lack sharp edges. Theoretically, this metric can be applied to model mismatches caused by other inaccurate experimental parameters as well, provided that system maintains a sufficient level of coherence. If the coherence drops too low, the optimization of the purity becomes unreliable. Additionally, we have introduced the concept of axial tolerance and demonstrated numerically and experimentally that the axial tolerance does not need to be on the order of the depth of field range. Instead, a more relaxed criterion is sufficient, significantly reducing the processing time, typically by several tens of minutes.

Summary and outlook. We proposed a self-calibration method based on purity. This method has been tested for axial distance calibration on synthetic and experimental datasets. In addition, due to the equivalence of axial distance and illumination wavelength in the diffraction integral, this method can also be employed to calibrate the wavelength uncertainty, preferably suitable for various applications in ptychographic experiments. We believe that the purity metric's reliance on system coherence makes it broadly applicable to computational imaging techniques that reconstruct phase and amplitude from intensity measurements, such as propagation-based phase contrast microscopy. Future work will focus on accelerating the

code, incorporating a self-adjusted-step purity scan, as well as implementation on reflection-mode ptychography.

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Data availability. Data underlying the results presented in this paper are available in Ref. [34].

Supplemental document. See Supplement 1 for supporting content.

REFERENCES

1. J. M. Rodenburg and H. M. L. Faulkner, *Appl. Phys. Lett.* **85**, 4795 (2004).
2. A. M. Maiden and J. M. Rodenburg, *Ultramicroscopy* **109**, 1256 (2009).
3. J. M. Rodenburg and A. Maiden, in *Springer Handbook of Microscopy: Springer Handbooks* (Springer, 2019).
4. P. Thibault, M. Dierolf, A. Menzel, *et al.*, *Science* **321**, 379 (2008).
5. F. Pfeiffer, *Nat. Photonics* **12**, 9 (2018).
6. B. L. Henke, E. M. Gullikson, and J. C. Davis, *At Data Nucl. Data Table* **54**, 181 (1993).
7. D. A. Shapiro, Y.-S. Yu, T. Tyliczszak, *et al.*, *Nat. Photonics* **8**, 765 (2014).
8. L. Loetgering, M. Rose, K. Keskinbora, *et al.*, *Opt. Eng.* **57**, 084106 (2018).
9. M. Guizar-Sicairos and J. R. Fienup, *Opt. Express* **16**, 7264 (2008).
10. A. M. Maiden, M. J. Humphry, M. C. Sarahan, *et al.*, *Ultramicroscopy* **120**, 64 (2012).
11. E. H. R. Tsai, I. Usov, A. Diaz, *et al.*, *Opt. Express* **24**, 29089 (2016).
12. J. Dou, Z. Gao, J. Ma, *et al.*, *Opt. Laser Eng.* **98**, 56 (2017).
13. L. Loetgering, M. Du, K. S. E. Eikema, *et al.*, *Opt. Lett.* **45**, 2030 (2020).
14. T. Ruan, W. Lv, Y. Tao, *et al.*, *Opt. Laser Eng.* **158**, 107136 (2022).
15. Z. Pei, Y. Hu, Z. Yang, *et al.*, *Optik* **286**, 171026 (2023).
16. M. Odstrcil, P. Baksh, S. A. Boden, *et al.*, *Opt. Express* **24**, 8360 (2016).
17. P. Thibault and A. Menzel, *Nature* **494**, 68 (2013).
18. A. de Beurs, L. Loetgering, M. Herczog, *et al.*, *Opt. Lett.* **47**, 1949 (2022).
19. J. Seifert, D. Bouchet, L. Loetgering, *et al.*, *OSA Continuum* **4**, 121 (2021).
20. Y. Shao, S. Weerdenburg, J. Seifert, *et al.*, *Light: Sci. Appl.* **13**, 196 (2024).
21. M. A. Alonso, *Adv. Opt. Photonics* **3**, 272 (2011).
22. R. Röhrich, A. F. Koenderink, S. Witte, *et al.*, *New J. Phys.* **23**, 053016 (2021).
23. B. Enders, "Development and application of decoherence models in ptychographic diffraction imaging," Ph.D. thesis (Technical University Munich, 2016).
24. A. Maiden, D. Johnson, and P. Li, *Optica* **4**, 736 (2017).
25. E. Wolf, *Opt. Commun.* **38**, 3 (1981).
26. L. Mandel and E. Wolf, in *Optical Coherence and Quantum Optics* (Cambridge University Press, 1995).
27. L. Loetgering, M. Du, D. Boonzajer Flaes, *et al.*, *Opt. Express* **31**, 13763 (2023).
28. R. Klas, W. Eschen, A. Kirsche, *et al.*, *Opt. Express* **28**, 6188 (2020).
29. M. Guizar-Sicairos, M. Holler, A. Diaz, *et al.*, *Phys. Rev. B* **86**, 100103 (2012).
30. W. Eschen, C. Liu, D. S. Penagos Molina, *et al.*, *Opt. Express* **31**, 14212 (2023).
31. C. Liu, W. Eschen, L. Loetgering, *et al.*, *Photonix* **4**, 6 (2023).
32. M. W. M. Jones, K. Elgass, M. D. Junker, *et al.*, *Sci. Rep.* **4**, 6796 (2014).
33. W. Eschen, L. Loetgering, V. Schuster, *et al.*, *Light: Sci. Appl.* **11**, 117 (2022).
34. C. Liu, D. S. Penagos Molina, and J. Rothhardt, "Raw ptychographic synthetic data for manuscript with title 'Purity-based self-calibration in ptychography'," Zenodo (2024), <https://doi.org/10.5281/zenodo.13773111>.