An autofocusing algorithm for ptychography is proposed. The method optimizes a sharpness metric that would be observed in a differential interference microscope and is valid for both amplitude and phase modulating specimens. We experimentally demonstrate that the algorithm, based on the extended ptychographic iterative engine (ePIE), calibrates the sample–detector distance with an accuracy within the depth of field of the ptychographic microscope. We show that the method can be used to determine slice separation in multislice ptychography, provided there are isolated regions on each slice of the specimen that do not axially overlap.

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Axial misalignment in the sample–detector distance results in scan grid miscalibration. The black coordinates (dashed line) illustrate an inflated scan grid as compared to the true encoder positions (gray coordinates, solid line). The depicted situation arises when underestimating the sample–detector distance, while an overestimation would result in a compressed scan grid.

Algorithm 1. Axial position correction algorithm (zPIE)

1: Procedure zPIE (P, O, z)
2: \( \delta z \leftarrow 0 \)
3: \( \eta \leftarrow 0.7 \)\( \triangleright \eta \): damping constant
4: \( c \leftarrow 1 \)\( \triangleright c \): step size
5: for \( m \leftarrow 1 \) to \( n \) do
6: \( (P, O) \leftarrow \text{ePIE}(P, O, z) \)
7: for \( k \leftarrow -K/2 \) to \( K/2 \) do
8: \( s(k) = S(F^{-1}H_{0,\Delta z}F O) \)
9: \( \delta z \leftarrow \eta \delta z + c \sum_{k} \frac{s(k)}{s(0)} - s(k) \)
10: \( z \leftarrow z - \delta z \)
11: return \( P, O, z \)

\[ \Delta z = \lambda (2z/D)^2. \] (4)

The sharpness of the propagated object is evaluated using the total variation (TV) functional

\[ S(z) = \iint \left( |\partial_x O(x, y, z)|^2 + |\partial_y O(x, y, z)|^2 + \epsilon dx dy \right) \],

\[ S(z) \approx S(z^*) + (z - z^*) \frac{\partial}{\partial z} S(z^*) + \frac{1}{2} (z - z^*)^2 \frac{\partial^2}{\partial z^2} S(z^*), \] (6)

where \( z^* \) is an arbitrary point. At the optimum \( z^* \), the first-order term vanishes:

\[ S(z) \approx S(z^*) + \frac{1}{2} (z - z^*)^2 \frac{\partial^2}{\partial z^2} S(z^*). \] (7)

Since \( S(z) \) is symmetric around \( z^* \) to second order, the feedback term \( \sum_k s(k) \Delta z/\sum_k s(k) \) in Algorithm 1 is zero at the optimum. For non-optimal \( z \), the skewness of the Taylor series expansion of \( S(z) \) can be used to compute a feedback on \( z \). In addition to the feedback term, the search direction \( \delta z \) has a damped momentum term \( \eta \), which allows the algorithm to accelerate the search in the case of repeated steps in the same direction [21]. We note that the search can be accelerated by increasing the proportionality factor \( c \) in front of the feedback term in Algorithm 1. However, this can potentially result in less numerical stability and overshooting around the optimum. All results reported here were obtained with \( K = 10, c = 1, \) and \( \eta = 0.7 \). Last, we note that the ePIE subroutine in Algorithm 1 depends on \( z \), which implies resampling the scan grid according to Eq. (1).

We tested the performance of the autofocus method using the experimental setup depicted in Fig. 2. A supercontinuum laser spectrally filtered to a wavelength of \( \lambda = 708.9 \text{ nm} (\Delta \lambda = 0.6 \text{ nm}) \) was spatially filtered and focused to illuminate a sample mounted on an \( xy \) translation stage (2× Smaract SLC-1770-D-S). The sample–detector distance \( z \) was set to be approximately 35 mm. An AVT Prosilica CCD camera (1456×1936 pixels with pixel size of 4.54 µm) was used to record a set of 200 diffraction intensities downstream the specimen. The average linear overlap in the scan was around 80% [22] at a beam size (FWHM) of 572 µm. We used a USAF resolution target (Thorlabs R3L1S4P) as a test sample, as it allows to compare the physical dimensions of the reconstructed image with the nominal dimensions according to the manufacturer.

In this way, we validate the retrieved sample–detector distance \( z \), assuming \( \lambda \) and \( D \) are known. The experimental results are shown in Fig. 3. Figures 3(a) and 3(b) show an object reconstruction obtained with an initial estimate of \( z_0 = 35.5 \text{ mm} \) and using zPIE. The smallest resolved feature, group 7/element 1, has a line width of 3.9 µm, indicating a half-period spatial resolution close to the diffraction limit of 3.8 µm. The line widths of group 4/element 1 have a size of 31.1 µm, which deviates less than 1% from the nominal value of 31.3 µm. In contrast, Figs. 3(c) and 3(d) show a reconstruction obtained with an initial estimate of \( z_0 = 35.5 \text{ mm} \) without using zPIE. While for the larger spatial structures the reconstruction quality is comparable to Fig. 3(a), the reconstructed high-resolution features suffer from an incorrect sample–detector distance estimate, as seen by comparing Figs. 3(b) and 3(c). Figure 3(e) shows the estimated \( z \) as a function of iteration. Here we varied the initial estimate of the sample–detector distance from 30 mm to 40 mm and used zPIE to find the correct value of \( z \). For small excursions around...
with autofocus versus iteration with varying initial estimates \( z_0 \).

(a), (b) correspond to the central dotted-dashed line in (e).

Fig. 3. Ptychographic reconstruction (a), (b) with autofocus and (c), (d) without autofocus using an initial object–detector distance of \( z_0 = 35.5 \) mm. (e) Estimated sample–detector distance \( z \) versus iteration with varying initial estimates \( z_0 \). (a), (b) correspond to the central dotted-dashed line in (c).

We carried out a second experiment to test the performance of zPIE in recovering the slice thickness of a thick substrate. To this end, we turned around the USAF resolution target and used zPIE to estimate the new sample–detector distance. From the difference of the estimated sample–detector distances of the experiments with the sample facing the detector and the sample flipped around, the substrate’s thickness can be inferred provided its refractive index is known. The substrate of the USAF resolution target has a geometrical thickness of 1.5 mm and a refractive index of \( n = 1.52 \) (soda lime glass) at a wavelength of 708.9 nm. On the front and back sides facing the illumination and detector, respectively. The glass slide (Thermo Scientific, Menzel Gläser) has a geometrical thickness of 1 mm and a refractive index of 1.52 at a wavelength of 708.9 nm. On the front and back sides of the slide, fingerprints were placed such that there were both overlapping and non-overlapping regions in the axial direction. First, we carried out single-slice experiments on the axially non-overlapping regions, using zPIE to recover the thickness of the glass substrate. This resulted in a (geometrical) thickness of \( t = 1.04 \pm 0.04 \) mm, where the uncertainties are estimated by repeating the experiment at different locations. A value 1.52 times lower was used in the 3PIE algorithm [15] to emulate the equivalent free-space distance between the individual slices. The results of the multislice reconstruction are shown in Fig. 5. Figures 5(a) and 5(b) show the recovered object amplitude using single- and multislice ptychography, respectively. Figure 5(b) shows the product of the individual object amplitudes \( \prod_j |O_k| \). Comparing the amplitude reconstructions in Figs. 5(a) and 5(b) indicates that the single-slice model exhibits inferior spatial resolution in recovering the back-slice of the specimen. Figure 5(c) shows the reconstructed probe, which is a 500 \( \mu \)m diameter pinhole with Scotch Tape stuck on top of it and imaged onto the backside of the specimen. The Scotch Tape generates a highly structured beam with increased spatial frequency content, resulting in reduced dynamic range requirements on the detector [15,23–26]. Figure 5(d) shows the individual reconstructed slices obtained by 3PIE overlain in green (front) and red (back).

The results reported here show that zPIE is a useful calibration tool for ptychography. For single-slice ptychography, we used a USAF resolution target to verify that the algorithm reconstructed the correct pixel size and thereby the correct sample–detector distance. Flipping the sample around and knowing the refractive index \( a \) priori, zPIE allowed us to measure the USAF target’s substrate thickness. For the multislice
experiments, a biological multislice specimen was chosen to show both that the sharpness metric in Eq. (5) holds for mixed amplitude and phase specimens and that zPIE can be used to calibrate slice separation in multislice ptychography. With regard to the latter, we investigated a specimen that contained small features of size $x_0$, which diffract under axial displacement $\Delta z$. In the case of both 3D specimens where multiple slices obscure each other and smooth 2D specimens, small calibration markers at the lateral resolution limit of the system may be added to the side of the specimen to calibrate the sample–detector distance. Furthermore, zPIE may serve as a useful tool in particular for multislice near-field ptychography [25]. As has been noted previously [15], in single-slice ptychography an imprecise knowledge of the quadratic phase in the integrand of the Fresnel diffraction integral can be absorbed into the probe. In contrast, in multislice ptychography, this is not the case due to scattering from multiple slices of the sample. In summary, we have shown an autofocusing algorithm for ptychography that allows to calibrate the sample–detector distance and the slice separation in single- and simple multislice specimens. We expect the method to find application in automated calibration applications for psychographic scanning microscopes.

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