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Off-axis and inline electron holography: experimental comparison

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Electron holography is a very powerful technique for mapping static electric and magnetic potentials down to atomic resolution. While electron holography is commonly considered synonymous with its off-axis variant in the high energy electron microscopy community, inline electron holography is widely applied in low-energy electron microscopy, where the realization of the off-axis setup is still an experimental challenge. This paper demonstrates that both inline and off-axis holography may be used to recover amplitude and phase shift of the very same object, in our example latex spheres of 90 and 200 nm in diameter, producing very similar results, provided the object does not charge under the electron beam.

This paper is dedicated to Prof. Hannes Lichte on the occasion of his 65th birthday.
1 Introduction

The experimental setup for electron holographic recording, as originally proposed by Dennis Gabor [1,2] considered the information contained in the interference pattern formed between the scattered and unscattered electron beam and would therefore be called inline holography. The first electron hologram was recorded in 1952 by Michael E. Haine and Tom Mulvey under the supervision of Dennis Gabor by using Gabor’s inline holography scheme with 60 keV energy electrons. They reconstructed it optically and achieved a resolution of about 10 Å[3].

An intrinsic feature of all types of holography is the presence of the twin image. The illustration of the position of the twin images in in-line and off-axis holography is shown in Fig. 1a. The twin images overlap spatially in the case of inline holography, see Fig. 1a. Already in his original work, Dennis Gabor discussed possible experimental solutions to spatially separate the twin images problem by using beam-splitters but concluded that this might be possible in light- but not electron optics, due to the lack of ”effective beam splitting devices” [2]. Shortly after the invention of the laser, not being aware of Gabor’s original work, Emmett N. Leith and Juris Upatnieks re-discovered holography but were able, because of the availability of beam-splitting light-optical devices, to set up an off-axis geometry in which the twin images could easily be separated [4,5]. In off-axis holography where the object is focused in the detector plane, as in case of high-energy off-axis electron holography, the reconstructed waves are diffracted in three different directions and the object wave can be selected by using an appropriate aperture in diffraction space, shown in Fig. 1b.

In an attempt to solve the twin image problem in the inline scheme, DeVelis et al. recorded holograms of an object in the Fraunhofer-diffraction plane [6]. In Fraunhofer holography, the distances between the object and its conjugated image are so
Figure 1. Position of object and its twin (in gray) during the recording and the reconstruction processes. The arrows show the direction of the incident beams. (a) Inline scheme with divergent spherical waves - original Gabor’s scheme. (b) Off-axis scheme applied in high-energy electron holography, the object is focused in the detector plane.

large, that the twin image is completely blurred out. Using Fraunhofer holography, Akira Tonomura at Hitachi Laboratory, a pioneer in the field of high-energy (off-axis) electron holography, also recorded electron inline holograms of fine gold, zinc oxide and
magnesium oxide particles in 1968 [7]. Besides Akira Tonomura (see a review of his work refs. [8] and [9]), also Gottfried Möllenstedt, the original inventor of the electrostatic biprism [10,11], and later Hannes Lichte explored numerous applications of off-axis electron holography (see, for example, [12,13]). With the invention of extremely sharp tips as field emission sources acquiring inline holograms with coherent low-energy electrons became possible [14,15]. 60 – 200 eV low-energy electrons have proven to cause the least if any radiation damage to biological samples such as fragile DNA molecules [16]. A low-energy electron off-axis holography, combining off-axis and inline point projection electron holography was proposed and realized by Pierre Morin in 1996 [17,18].

Initially, holograms were reconstructed optically. With the fast development of computers, numerical reconstruction of holographic images became possible [19,20]. While the digital reconstruction of off-axis holograms was a rather direct translation of the optical bench setup into a digital image processing routine, for inline holography this development provided a chance to finally solve the twin image problem. A number of different approaches to the reconstruction of inline holograms have been proposed. Most of the numerical routines used for the reconstruction of single inline holograms are based on the idea of using an iterative reconstruction from measured intensity distributions as suggested by Ralph W. Gerchberg and Owen W. Saxton in 1972 [21] using so-called support functions, which can be some physical a priori knowledge of the object shape or some mathematical constraint on the object transmission function [22,23]. Alternative reconstruction schemes, which require recording inline holograms at more than just one plane of defocus are based on the (infinitesimal) change of the image intensity with defocus ((TIE) [24]) or a fit of simulated defocused images to an experimental defocus series [25] using either linear or non-linear approximations to the imaging process.

Both schemes, inline and off-axis holography, have been widely
implemented in optical and electron holography. Direct comparisons of the different techniques, however, are rare. Twitchett et al. [26] applied off-axis and what they called ”inline holography” to study the electrostatic potential of a silicon $p$-$n$-junction. They found good agreement between the results obtained by zero-loss energy-filtered Fresnel images and off-axis holography, if a constant background attributed to diffuse scattering and proportional to the sample thickness is subtracted from the defocus series of Fresnel images. It should be noted here, that the original definition of holography by Gabor [2] is that of a two-step process of first recording the hologram and then reconstructing the complex wave function from the experimental data. Looking for agreement between experimental and simulated Fresnel images is therefore, strictly speaking, not ”inline holography”. The off-axis holograms allowed an accurate determination of the step in phase shift across the $p$-$n$-junction while the Fresnel images provided a higher spatial resolution around the junction. Watson et al. [27] designed a unique camera which allowed to record simultaneously both inline and off-axis optical holograms of marine particles. They also found that off-axis holograms reveal the true three-dimensional structure of objects while the reconstruction of inline holograms provides higher resolution.

In the following sections we demonstrate high-energy electron holography of the same object - a latex sphere, recorded in both, inline and off-axis scheme. This has the advantage that the geometry of the specimen is well defined (no modified layers due to FIB preparation) and the diffraction contrast is very small. The off-axis holograms were reconstructed using the usual reconstruction scheme [28], with some reconstruction steps improved [29]. Two reconstruction schemes were used for phase retrieval from inline holograms: reconstruction from a single inline hologram and reconstruction from a defocus series according to [30].
2 Experimental

The TEM specimens of latex spheres were prepared by putting a drop of suspension (diluted 10 times by distilled water) on a copper grid with holey carbon film (S147-3 by Plano GmbH, Germany). After solvent evaporation, spheres were distributed across the carbon film, see Fig. 2a. The first object (Sphere1) was a polystyrene latex sphere of 204 nm diameter (S130-4, Plano GmbH). Since the Sphere1 exhibited charging (see Fig. 3b) as shown by the phase shift (the brighter halo) in the vacuum around the particle, a second specimen (Sphere2) was prepared in the same way using 112 nm spheres (S130-1, Plano GmbH). The second specimen was coated with 4 nm amorphous carbon prior to investigation to reduce the charging.

A Phillips CM200 FEG microscope was used for recording holograms. The off-axis holograms were recorded using the Lorentz objective lens to achieve hologram widths containing the whole object. In the off-axis regime, see Fig. 2b, the biprism is placed in front of an intermediate image plane to superimpose object wave and reference wave in the image plane. The biprism voltage was between 130 V and 140 V yielding a fringe spacing between 3.86 nm and 4.09 nm.

The inline holograms were recorded using the SuperTwin objective lens in order to avoid the very large aberrations of the Lorentz lens. For inline holograms, the defocus was controlled by shifting the specimen stage in z-direction, see Fig. 2c and the holograms are recorded at some \( \Delta f \) defocus distance from the focal plane. The accuracy of the stage is 0.5 micrometer (as specified by the microscope manufacturer) and the readout of the stage position was used as an initial value for the defocus value which was determined more exactly within the reconstruction process.

All holograms were acquired using a 1024 \( \times \) 1024 pixels 16 bit Gatan CCD camera (model 794).
Figure 2. An illustration to the experiment. (a) Overview image of the sample - polystyrene spheres on carbon net. The red arrow shows Sphere2 selected for the holographic imaging. The black line in the bottom left corner is the shadow of the biprism. (b) and (c) Drawings of holographic off-axis and inline schemes. The red (blue) color represents the object (reference) wave. $\Delta f$ is the defocus distance.

3 Reconstruction of electron holograms

3.1 Reconstruction of off-axis holograms

The numerical reconstruction of off-axis holograms consists of two mathematical transformations [28]. First, a Fourier transform of the hologram is performed. The resulting complex image consists of the autocorrelation (center band) and two mutually conjugated sidebands. In the complex image only one sideband is selected by applying a low-pass filter centered on the chosen sideband, which damps both the central band and the other sideband to zero. Subsequently, the sideband is centered in Fourier space with sub-pixel precision and transformed back into real space.

For quantitative off-axis holography measurements, directly after recording the object hologram a second hologram without the object is acquired (empty hologram). This empty hologram is later used to correct distortions in the reconstructed wave stemming from an unevenly charged biprism, geometric distortions of the
projective lens and the fiber optics of the camera [28]. These distortions in the reconstructed object and empty wave can be described by a position dependent phase offset. By dividing the object wave with the empty wave this phase offset is readily removed. As a side effect the object wave is normalized and remaining phase wedges due to an incorrect sideband centering are removed. Note, however, that the quotient of two with noise afflicted quantities (the reconstructed waves) leads to a noise amplification, which corresponds roughly to a multiplication of the standard deviation by a factor of $\sqrt{2}$ in case of the reconstructed phases (sum of two independent normally distributed variables).

Figure 3. Off-axis hologram of Sphere1 and its reconstruction. (a) Off-axis hologram of Sphere1. The Fresnel fringes from the biprism filament edge are readily visible. (b) Reconstructed amplitude. Note that the Fresnel contrast is drastically reduced. (c) Unwrapped reconstructed phase with a gray map corresponding to phase values between 0 and 13.16 rad.

Figure 4. Off-axis hologram of Sphere2 and its reconstruction. (a) Off-axis hologram of Sphere2. (b) Reconstructed amplitude of the object wave. (c) Unwrapped reconstructed phase with a gray map corresponding to phase values between 0 and 6 radian.
Additionally, both the object hologram and the empty hologram have been prepared for the actual reconstruction according to the following procedure: First, very bright and dark pixels as produced by X-rays hitting the detector and dead pixels on the CCD chip are replaced by surrounding pixel values [31]. After that, the holograms are Fourier transformed and divided by the modulation transfer function (MTF) of the camera. The deconvolution of the MTF increases the fringe contrast measured in the vacuum. In the example shown in Fig. 3 the contrast increased from originally 8% to 23%. As the large hologram width used in the experimental setup contains Fresnel fringes (see Fig. 3a and Fig. 4a) produced by the sharp edge of the biprism filament, which introduce additional phases and amplitudes in the reconstructed wave not stemming from the specimen, an additional preprocessing step consisting of a numerical subtraction of the Fresnel fringe contrast was introduced.

3.2 Focal series reconstruction of inline holograms (FSR)

Focal series reconstruction techniques try to reconstruct an electron wave function, which is able to predict a set of inline holograms recorded at different planes of defocus. The larger the range of defocus over which the reconstructed wave function is able to match the experimentally obtained image contrast the more reliable is the reconstruction algorithm.

The iterative reconstruction algorithm applied in this work is based on the following flux-preserving expression for the intensity of the defocused images [30]:

\[
I(\vec{r}, \Delta f) = \left| \text{FT}^{-1} \left[ \Psi_0(\vec{q}) \exp(-i\chi(q)) E_t(\Delta f, q) \right] \right|^2 \\
\otimes \text{FT}^{-1} \left[ E_s(\Delta f, \alpha, q) \right],
\]

(1)

where
\[
\chi(q) = \pi \lambda \Delta f q^2 + 0.5\pi \lambda^3 C_s q^4
\]
\[
E_t(\Delta f, q) = \exp \left( -\Delta f^2 \left[ \frac{\partial \chi(\vec{q})}{\partial \Delta f} \right]^2 \right)
\]
\[
E_s(\Delta f, \alpha, q) = \exp \left[ - (\pi \alpha \Delta f q)^2 \right]
\]

Here \( \Delta f \) is the defocus, \( \Psi_0(\vec{q}) \) - the exit face wave function in reciprocal space, \( \Delta f \) is the focal spread, \( \lambda \) is the electron wavelength, \( \alpha \) is the illumination semiconvergence angle, \( C_s \) - the spherical aberration of the objective lens, and \( E_t \) and \( E_s \) the temporal and spatial coherence envelopes.

The above approximation can be derived from the formulation involving the transmission cross coefficient (TCC) [32] by assuming that the effects of the spherical and other aberrations are negligible compared to that of the objective lens defocus (see also [33]). The details of the reconstruction algorithm have in most part already been described in reference [30]. At each iteration the image intensities at the different planes of defocus are simulated from a trial wave function, the difference between simulated and experimental image amplitude is added to the amplitude of the simulated wave, and a new trial wave function is generated by averaging the updated back-propagated wave functions.

Due to the change of the defocus by adjusting the position of the specimen, images recorded at different defoci are rotated with respect to one another, have a slightly different magnification, and may also have been subject to spiral or pincushion distortions. Between iterations of the reconstruction algorithm the optimum values for these distortions are therefore fitted by comparing differently distorted versions of the simulated image with the experimental image at the same defocus.

Likewise, the exact defocus of each image is being determined by comparing images simulated for different defocus values with the experimental ones. For sufficiently good initial estimates of the actual defocus values, the massively overdetermined set of non-
Figure 5. (a), (c), (e), (g), and (i) Experimental inline holograms of Sphere1 recorded at different values of the defocus. (l) and (m) show the Amplitude and Phase of the object wave function reconstructed by an iterative flux-preserving focal series reconstruction algorithm. The grey scale in the phase map corresponds to values of the phase between 0 and 14 rad. The gray levels in the amplitude image correspond to 40 ... 105 % of the amplitude in the vacuum region. Images (b), (d), (f), (h), and (k) have been simulated from the reconstructed wave function for the defocus values used to record the experimental data (gray scale: 12 ... 278 % of intensity in vacuum region).

Linear equations which map the intensity of the different inline holograms to a single complex-valued wave function and a few defocus values ensure that the determination of all the unknowns is unique. Iterative refinement of the defocus values worked even in the case presented in Fig. 5, where both the experimental inline holograms as well as the corresponding images at each defocus value are shown. In this example the very large defocus and the strong circular features in the diffractogram caused by the Fresnel fringes around the latex sphere made it impossible to identify the defocus of each image by identifying zeros in the contrast transfer function.
function.

Comparing the experimental and simulated inline holograms in Fig. 5 it is obvious that the agreement is not perfect. The discrepancy can be quantified by the $R$-factor [34]

$$R = \frac{1}{N_{\text{img}}} \sum_{j=1}^{N_{\text{img}}} \frac{\int \int |I_{\exp}(x, y, \Delta f_j) - I_{\sim}(x, y, \Delta f_j)| \, dx \, dy}{\int \int I_{\exp}(x, y, \Delta f_j) \, dx \, dy} \quad (5)$$

The $R$-factor for the focal series reconstruction of Sphere1 was 4.5%, which, despite of some small differences indicates still a very good agreement.

Since the diameter of Sphere2 is only about half that of Sphere1 the defocus step in the second example was chosen much smaller than in the first example (18 $\mu$m instead of 200 $\mu$m). Figure 6 shows the focused and defocused images as well as amplitude and phase of the wave function reconstructed from it. The absolute defocus of the two images was determined from the position of the rings of vanishing contrast transfer (thin rings) in the power spectrum of the images and was not fitted during the reconstruction.

### 3.3 Iterative reconstruction from a single inline hologram (SIR)

To be able to obtain a reconstruction from a single inline hologram, a second inline hologram without the object is required (empty hologram). The empty hologram provides the distribution of the amplitude of the reference wave. In some experimental cases, however, it is difficult to record the second hologram of exactly the same area with the exactly the same illumination, but without object (for instance, due to the electron source electrical or mechanical instabilities). In such cases, the empty hologram is created numerically by simple low-pass filtering of the hologram of the object. The filtering removes all the interference fringes caused by the presence of the object.
Figure 6. (a) - (b) Inline holograms of Sphere2 recorded at different values of the defocus. (c) and (d) amplitude and phase of the object wave function reconstructed by the iterative flux-preserving focal series reconstruction algorithm. The grey scale in the phase map corresponds to values of the phase between 0 and 5 rad. The contrast levels of the amplitude map are between 40 and 120 % of the amplitude in the vacuum region. The R-factor for this reconstruction was 4%.

In the next step, the minimal value of the intensity is subtracted from both, the hologram of the object and the empty hologram. Then, the hologram of the object is divided with the empty hologram. This normalization aims to make use of the known distribution of the reference wave in order to extract the absorption and the phase shifting properties of the object [35].
The following parameters are used in the reconstruction: wavelength $\lambda = 2.51\ \text{pm}$ for electron energy $E = 200\ \text{keV}$, and the image size - 900 nm×900 nm for the first sample and 450 nm×450 nm for the second sample. As an initial step, the inline hologram is reconstructed by simple backward propagation [36] for different $\Delta f$ distances. The distance where the object appears as the best reconstructed $z'\prime$ is selected for the iterative reconstruction procedure. It was found that for both Spheres, the best in-focus reconstruction distances are equal to the defocus distance of the SuperTwin lens: $z'\prime = -180\ \mu\text{m}$ for Sphere 1 and $z'\prime = -60\ \mu\text{m}$ for Sphere 2.

The iterative reconstruction algorithm consists of the following steps:

(i) Backward propagation from the detector plane to the object plane.

(ii) The absorption and the phase of the object are extracted and constrains are applied. The filtered absorption and phase distributions are recombined into an updated transmission function.

(iii) Forward propagation to the hologram plane.

(iv) The argument of the propagated wave is set as an updated phase and the square root of the measured intensity is set as the amplitude of the field in the detector plane. The updated field in the detector plane is an input function for the next iteration.

In the iterative procedure the forward and backward propagations between the object and the screen plane are calculated using the angular spectrum method [36].

Since the polystyrene spheres are simply attached to a lacey carbon support in vacuum the following constraint on the electron wave function seems reasonable - the transmission should be 1 outside the area occupied by sphere or carbon film. Numerically, the retrieved object transmission function is multiplied with a
mask image, see Fig. 7a and Fig. 8a, at each iteration. For the first 300 iterations the constraint of non-negativity [37] and a smoothing filter are applied to the reconstructed absorption distribution - this helps to suppress the fringy structure in the reconstructed image significantly and to obtain the rough shape of the object.

During the entire reconstruction procedure, the phase of the wave function in the object plane remains unconstrained. In addition, at each iteration the phase distribution is unwrapped [38] to control the phase retrieval visually. The overall number of iterations is about 4000. The retrieved amplitude and phase distributions of the exit object wave are shown in Fig. 7(b,c) and Fig. 8(b,c).

Figure 7. Inline hologram of Sphere1 and its reconstruction. (a) Inline hologram of Sphere1 recorded with SuperTwin lens at the defocus $-180 \mu m$. The blue line marks the areas outside which the transmission is set to 1. (b) Retrieved amplitude distribution of the object wave. (c) Retrieved phase distribution of the object wave. Maximum of the phase shift is about 14 radians.
Figure 8. Inline hologram of Sphere2 and its reconstruction. (a) Inline hologram of Sphere2 recorded with at the defocus $-60 \mu m$. The blue line marks the areas outside which the transmission is set to 1. (b) Retrieved amplitude distribution of the object wave. (c) Retrieved phase distribution using support function. Maximum of the phase shift is about 6 radians.

4 Discussion

In the following we will conduct a quantitative comparison of the holographic phase maps obtained by the different methods. We will now discuss the two examples, off-axis and inline holography of charging and non-charging latex spheres.

4.1 Charged polystyrene sphere (Sphere1)

In the first example the holograms were acquired from an uncoated latex sphere, Sphere1, which collected positive charge under the electron beam. This charge produced a positive potential "hill" around the latex sphere, raising the overall potential of the sphere as well. There is obvious charging of the sphere recorded with off-axis holography which manifests as tails in the reconstructed phase profiles in the vacuum region around the sphere (see Fig.9b). The tails in the reconstructed in-line holograms might be either charge or reconstruction artefacts or both.

Figure 9a shows the model that has been set up to fit the different phase profiles across the sphere. The phase profiles were fitted with function corresponding to a charged homogenous sphere with a certain mean inner potential. The charge was modeled as
Figure 9. Fitting a charge distribution to phase profiles extracted from off-axis and inline holograms. (a) Within the model used to produce the solid lines in (b) - (d) the projected potential is the integrated along the electron trajectory for each beam position. In order to avoid divergence of the potential a compensating charged sphere with a very large radius had to be placed around the latex sphere. (b) - (d) experimental (dots) and fitted (solid line) phase shifts.

a uniform volume charge with a total charge $Q$. In order to remove the divergent part of the projected potential and thus make the potential integrable, a charged spherical shell of radius $P$ and total charge $Q$ was added, with $P \gg R$. In addition to the total charge of the sphere the following parameters were fitted to the experimental data: $R$ - radius of the sphere (about 92 nm from a simple bright-field TEM image), $V_0$ - the mean inner potential of the sphere ($V_0$ of polystyrene is 8.5 V [39]). A fixed dielectric constant of $\varepsilon_r = 2.5$ [40] has been assumed inside of the sphere. For the fitting formulas see Appendix 1.

The comparison of the different experimental profiles with the
fitted ones shows that only the off-axis result truly represents the phase shift of a charged dielectric sphere. There may be several reasons for this.

The first argument is that the worse resolution of small frequencies in inline is rather connected to the incompletely blurred out twin image, which destroys information in Fourier space by convolution. The second argument is that because of the elliptical illumination the spatial coherence used for the off-axis experiments was much larger than in the inline case. This allows the reconstruction of very low spatial frequency information. The FSR phase map which has been reconstructed from 5 inline holograms recorded over a very large range of defocus conditions reproduces the mean inner potential of polystyrene but shows a very poor match of the charge-induced phase shift outside the sphere. Because of the change in defocus the electron flux density in the plane of the sample also changed between different inline holograms. It is therefore conceivable that each of the inline holograms represents a different amount of charge stored in and on the latex sphere. The assumption of the reconstruction algorithm of the inline holograms all representing the same complex-valued electron wave function is therefore violated.

Figure 10 shows the fitted amplitude distribution of Sphere1 reconstructed by the three different methods. The fitting formula is discussed in Appendix 2. The radius of the sphere is 98 nm, 96 nm and 100 nm for off-axis, FSR and SIR reconstructions, correspondingly, which accounts to the uncertainties of magnification. The inelastic mean free-path for polystyrene spheres imaged with 200 keV electrons was measured 113 nm in Ref.[41]. From the fitting of our experimental data we obtain the following mean free-path values - 123 nm, 281 nm and 106 nm for off-axis, FSR and SIR reconstructions, correspondingly. As it can be seen in Fig. 10, while the off-axis and SIR reconstructions show similar shape and the drop of the amplitude down to about 0.4, the FSR reconstruction shows the decrease of the amplitude to 0.7. This difference can be explained by the fact that the images were
not energy-filtered and the FSR algorithm does not include the contribution of inelastically scattered electrons.

4.2 Uncharged polystyrene sphere (Sphere2)

Figure 11 shows phase profiles and Fig. 12 shows the amplitude profiles across the center of Sphere2. The fitting formula is discussed in Appendix 2. From the fitting of the amplitude profiles we obtain the following mean free-path values - 128 nm, 375 nm and 190 nm for off-axis, FSR and SIR reconstructions, correspondingly. Two different off-axis holograms of the very same object have been reconstructed, each resulting in a slightly different phase shift. This difference may be due to a slight rotation of the sphere around the axis of the carbon support between successive holograms. Two different reconstructions from a single inline hologram (SIR) are also shown, each for a different assumed defocus. Since the focal series reconstruction (FSR) was done for the precise defocus determined from the position of the Thon rings in the power spectrum only a single reconstruction was performed.

The agreement between all 3 reconstruction techniques is quite good, although not perfect. As already mentioned some of the discrepancies may be attributed to changes in the projected potential of the object itself. Differences may also be due to the different signals detected by the off-axis and inline geometry. While off-axis holography very efficiently removes any incoherent contribution to the images, this is not the case for inline holography. To quantify this effect for off-axis holograms, the sideband intensity (absolute square of the reconstructed amplitude) was compared to the center-band intensity. In the vacuum region, the ratio between both was 1 (as expected), and it dropped to a constant value of approximately 0.5 within the sphere, indicating, that 50% of the electrons transmitting the sphere have been scattered inelastically.
Figure 10. Fitting the amplitude profiles across the center of Sphere1 obtained by the different reconstruction methods. Experimentally measured amplitudes are shown as dots, while the fitted amplitudes are shown as solid lines.
The contribution of incoherently scattered electrons to the experimental inline holograms is not considered in either of the two reconstruction methods applied here. This difference between theory and experiment is expected to produce a systematic error in the reconstruction. The phase profiles shown in Fig. 11 do not indicate a particular way how this affects the result. A comparison of the experimental inline holograms and those simulated from the reconstructed wave function may provide an indication of how focal series reconstructions may be affected. For example, the contrast in the experimental data in Fig. 5g is much smaller than the simulated counter part in Fig. 5h.

Since the iterative algorithms, such as the one described in ref.
Figure 12. (a) Amplitude profiles across the center of Sphere2 obtained by the different reconstruction methods. (b) - (d) Experimental measured (dots) and fitted (solid line) amplitudes.

[30], reconstruct the information in the phase slowly, the algorithm can easily reconstruct phase shifts greater than $2\pi$ without having to 'unwrap' the phase of a complex wave function. This is done by simply adding the change in the phase between the previous estimate and the current estimate to a separate array, which keeps track of the phase shift only. This explains the very large phase range of Fig. 5f of 14 rad without any phase jumps of $2\pi$ (see also Fig. 7).

However, in most cases it is also easily possible to unwrap the phase extracted from the complex wave function using phase unwrapping algorithms available in the literature (see, e.g. [38]), so that this "on the fly" phase unwrapping does not provide a
significant advantage.

5 Conclusion

We have compared phases and amplitudes of reconstructed off-axis and inline holograms recorded of the very same object. Both holographic schemes have been realized on the same 200 kV FEG TEM. While at low energies the construction of the off-axis setup employing an electrostatic biprims is very difficult to realize, leaving inline holography as the method of choice, this was not a problem at the high accelerating voltage employed here. The inline holograms were reconstructed by two different iterative reconstruction schemes, one of which only required a single inline hologram, while the other required at least two holograms.

Both inline and off-axis holography experiments were performed at a carbon coated and an uncoated latex sphere supported by a thin strand of carbon. While the reconstructions agreed rather well for the carbon coated sphere, they differed substantially for the uncoated one, which was charging under the electron beam. Reasons for this may be differences in the charge induced at the surface and in the interior of the latex sphere between exposures. Such charge differences may also be due to the different illumination conditions applied for the acquisition of off-axis (astigmatic illumination) and inline holograms (round illumination).

The conclusion to be drawn from this experimental comparison is thus: the phase shift of objects which are stable under the electron beam and do not change their charge distribution between exposures may be measured by off-axis as well as inline holography with similar precision. While the reconstruction of focal series requires at least two images recorded at different defocus, holographic techniques which require only a single image (off-axis holography or the single image reconstruction as applied in this work) may work better. However, both of the single hologram methods applied in this experimental comparison require a
hologram of empty space close to the object, which can be either measured experimentally or in some cases produced numerically from the hologram of the object.

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7 Appendix 1: Fitting the phase distribution of the charged polystyrene sphere

The electrostatic potential of the system of a dielectric charged sphere and an oppositely charged concentric spherical shell with a greater radius (Fig. 9, Section 4.1) can be calculated by dividing the space in 3 regions: outside of the spherical shell (potential \( V_0 \)), between the sphere and the shell (potential \( V_1 \)), and inside the sphere (potential \( V_2 \)) and applying the Gauss law in each region:

\[
V_0 = 0, \quad r \geq P, \quad (6)
\]

\[
V_1 = \frac{Q}{4 \pi \varepsilon_0 R} \left( \frac{R}{|r|} - \frac{R}{P} \right), \quad R \leq r \leq P, \quad (7)
\]

\[
V_2 = \frac{1}{4 \pi \varepsilon_0 R} \frac{1}{2 \varepsilon_R} Q \left( 1 - \frac{r^2}{R^2} \right) +
\]

25
\[
\phi(x) = C_E \int_{-\infty}^{\infty} V_0(x, y, z) \bigg|_{y=0} \, dz = 0.
\] (9)

At the distances \( R \leq |x| \leq P \):

\[
\phi(x) = C_E \int_{-\infty}^{z(A)} V_0(x, y, z) \bigg|_{y=0} \, dz + C_E \int_{z(B)}^{z(A)} V_1(x, y, z) \bigg|_{y=0} \, dz + C_E \int_{z(A)}^{z(B)} V_2(x, y, z) \bigg|_{y=0} \, dz + C_E \int_{z(A')}^{z(B')} V_1(x, y, z) \bigg|_{y=0} \, dz + C_E \int_{z(B')}^{z(A')} V_2(x, y, z) \bigg|_{y=0} \, dz =
\]

\[
= C_E K R \left( \ln \left( \frac{1 - \sqrt{1 - x^2/P^2}}{1 + \sqrt{1 - x^2/P^2}} \right) + 2\sqrt{1 - x^2/P^2} \right) Q. \] (10)

Inside the sphere \( |x| \leq R \):

\[
\phi(x) = C_E \int_{-\infty}^{z(A)} V_0(x, y, z) \bigg|_{y=0} \, dz + C_E \int_{z(A)}^{z(A')} V_1(x, y, z) \bigg|_{y=0} \, dz + C_E \int_{z(A')}^{z(B')} V_2(x, y, z) \bigg|_{y=0} \, dz + C_E \int_{z(B')}^{z(B)} V_1(x, y, z) \bigg|_{y=0} \, dz + C_E \int_{z(B)}^{z(B')} V_2(x, y, z) \bigg|_{y=0} \, dz =
\]

\[
+ \frac{1}{4\pi\varepsilon_0 R} Q \left( 1 - \frac{R}{P} \right), \ r \leq R,
\] (8)

where \( r = \sqrt{x^2 + y^2 + z^2} \).
\[ C_EKR \left( \ln \left( \frac{(1 + \sqrt{1 - x^2/R^2})(1 - \sqrt{1 - x^2/P^2})}{(1 - \sqrt{1 - x^2/R^2})(1 + \sqrt{1 - x^2/P^2})} \right) \right) Q \]
\[ + 2C_EKR \left( \sqrt{1 - x^2/P^2} - \sqrt{1 - x^2/R^2} \right) Q \]
\[ - \frac{2}{3\epsilon_r}C_EKP\sqrt{1 - x^2/R^2}Q, \quad \text{(11)} \]

where \( z(A) = \sqrt{P^2 - x^2} \), \( z(A') = \sqrt{R^2 - x^2} \), \( z(B') = -z(A') \), \( z(B) = -z(A) \) and \( K = 1/(4\pi\epsilon_0 R) \).

8 Appendix 2: Fitting the phase and amplitude distributions of the uncharged polystyrene sphere

The phase profiles were fitted with function corresponding to a homogenous sphere with a certain mean inner potential \( V \):
\[ \phi(x) = C_E V t(x). \]
\[ \phi(x) = C_E V 2\sqrt{R^2 - x^2} + C, \quad |x| < R, \quad \text{(12)} \]
\[ \phi(x) = C, \quad |x| \geq R, \quad \text{(13)} \]

where \( C_E = 7.28 \times 10^{-3} \) rad/V/nm, \( V \) - mean inner potential (initial value = 6 - 8 Volt), \( C \) - arbitrary constant, \( R \) - radius of the sphere.

The amplitude profiles were fitted with function corresponding to a homogenous sphere with a certain inelastic mean free path \( \lambda \):
\[ A(x) = A_0 \exp \left( -t(x)/(2\lambda) \right). \]
\[ A(x) = A_0 \exp \left( -\sqrt{R^2 - x^2}/\lambda \right), \quad |x| < R, \quad \text{(14)} \]
\[ A(x) = A_0, \quad |x| \geq R, \quad \text{(15)} \]
where $A_0$ is the amplitude of the reference wave (initial value taken from the vacuum region), $\lambda$ is the inelastic mean free path, $R$ is the radius of the sphere.
References


