Artifact characterization and reduction in scanning X-ray Zernike phase contrast microscopy

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Abstract: Zernike phase contrast microscopy is a well-established method for imaging specimens with low absorption contrast. It has been successfully implemented in full-field microscopy using visible light and X-rays. In microscopy Cowley's reciprocity principle connects scanning and full-field imaging. Even though the reciprocity in Zernike phase contrast has been discussed by several authors over the past thirty years, only recently it was experimentally verified using scanning X-ray microscopy. In this paper, we investigate the image and contrast formation in scanning Zernike phase contrast microscopy with a particular and detailed focus on the origin of imaging artifacts that are typically associated with Zernike phase contrast. We demonstrate experimentally with X-rays the effect of the phase mask design on the contrast and halo artifacts and present an optimized design of the phase mask with respect to photon efficiency and artifact reduction. Similarly, due to the principle of reciprocity the observations and conclusions of this work have direct applicability to Zernike phase contrast in full-field microscopy as well.

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1. Introduction

Scanning X-ray microscopy is a powerful tool for analyzing various types of samples due to the possibility of extracting several signals from a single measurement [1]. These signals include e.g. X-ray absorption [2], differential phase contrast [3], fluorescence [1], and emission of photoelectrons [4]. The fluorescence signal gives information on the chemical composition of the sample, but the fine structural information is not fully revealed. X-ray absorption is not sensitive enough in case of light elements and differential phase contrast imaging requires reconstruction to reveal the phase of the specimen. Zernike phase contrast (ZPC) with X-rays [5] is successfully applied in full-field microscopes for contrast enhancement in the case of weakly absorbing specimens. The Zernike phase contrast method is based on transferring the phase shift induced by the sample into intensity variations on the detector by applying a phase shift to the unscattered wavefront relative to the scattered one. The phase shift of the unscattered wave needs to be $\pm \pi/2$ for optimal contrast enhancement.

Implementing this method for scanning microscopy has been discussed by several authors [6, 7], but only recently it was applied in scanning X-ray microscopy [8]. The physical principle of the method has been explained by applying the reciprocity theorem for full-field Zernike phase

contrast microscopy that states that the roles of the source and detector can be interchanged [9]. In this paper, we present a more detailed description of the reciprocity in scanning Zernike phase contrast (SZPC) and explain qualitatively how the contrast and artifact formation can be described by exchanging the roles of the phase mask and the sample. In addition, we present the effect of the phase mask by simulations based on scalar diffraction theory. The effect of the phase shifting mask is experimentally demonstrated for various designs.

2. Image formation in Zernike phase contrast microscopy

The reciprocity principle in microscopy can be derived from the symmetry of the Fresnel-Kirchhoff diffraction integral [10]. For clarity, the phase shifter in the scanning geometry is from here on called phase mask and in the full-field case, phase plate.

2.1. Image formation in full-field Zernike phase contrast microscopy

In classical full-field microscopy a condenser lens illuminates a sample which is imaged onto a detector by an objective lens (OL) (see Fig. 1). Zernike phase contrast is enabled through a phase-shifting plate that matches the illumination in the conjugate plane of the condenser, i.e. the back focal plane of the OL. In the absence of a sample, the radiation coming from the condenser is imaged by the OL onto the phase plate and therefore all the incident radiation is phase shifted. The presence of a sample scatters part of the incident radiation coming from the condenser. The remaining unscattered part of the radiation is still imaged by the OL into the back-focal plane onto the phase plate and is shifted in phase. The scattered radiation does not get imaged onto the phase plate and therefore is not phase shifted. The two parts will interfere in the detector plane and form an image of the sample. The relative phase differences between the two parts and their separation in the back-focal plane (BFP) is the key element of Zernike's idea of phase contrast as it establishes sensitivity to the sample's phase shift information, which in absence of the phase difference between the two parts would be hardly detectable against the image background [11].



Fig. 1. Schematic illustration of the full-field Zernike phase contrast setup. Radiation arrives from the left and is focused to the sample by an annular condenser. The unscattered part (blue) is imaged onto the phase plate in the back-focal plane of the objective lens (OL) and interferes with the scattered part (gray) in the detector resulting in Zernike phase contrast. In case of annular condenser, the phase plate has a shape of a ring.

2.2. Image formation in scanning Zernike phase contrast microscopy

In SZPC microscopy (Fig. 2) a phase mask is placed upstream of the lens at a distance o and imaged through the lens onto a detector placed at a distance i such that the lens equation

1/o + 1/i = 1/f is fulfilled, where *f* is the focal length of the lens. Hence a magnified image of the phase mask is formed within the bright-field cone of the lens on the detector.



Fig. 2. Schematic illustration of the scanning Zernike phase contrast setup. Radiation arrives from the left, propagates through the phase mask, and is focused onto the sample with a lens. The subsequently transmitted radiation is collected by a pixelated detector that is required to enable integration support for the image of the mask.

In order to understand the image formation in SZPC microscopy we consider the following: the incident radiation is scattered in part by the phase mask, and part of the radiation remains unscattered. The unscattered radiation from the phase mask is focused to the focal spot of the lens, whereas the scattered light passes the focal spot within the focal plane and in essence contributes to the side-tails of the point spread function. This is analogous to the two spatially separated parts in the full-field case where the separation occurs in the back focal plane (BFP) of the imaging lens, i.e. in the plane of the phase plate. Now placing a phase shifting sample into the focal plane shifts the phase of the unscattered part compared to the scattered part from the phase mask. These two parts interfere in the detector plane leading to Zernike phase contrast. It follows that in SZPC microscopy the sample has the role of the Zernike phase shifter as it increases the contrast can be considered as full-field ZPC imaging of the phase mask with the sample in the focal spot as the phase plate. As the sample is scanned in the beam, increased contrast is visible for the imaged phase mask if the focal spot hits a phase shifting structure of the sample.

The image formation is described in Fig. 3. For each scanning point the Zernike phase contrast signal is computed by integrating the signal within the integration support defined by the full-field image of the phase mask (see Figs. 3(d) and 3(e)). This number becomes the value of the Zernike phase contrast signal in the respective scanning point, i.e. image pixel. Thus by simple analysis the method reveals the Zernike phase contrast image of the entire sample as shown Fig. 3(f). The resulting intensity variations between different scan points in the computed Zernike phase contrast signal is completely analogous to the full-field Zernike phase contrast image as demonstrated in [8].

In case of full-field Zernike phase contrast microscopy, the phase plate has to be designed according to the intensity pattern in the BFP of the objective lens. Therefore, it strongly depends on the illumination by the condenser [12, 13]. However, in case of SZPC microscopy the design of the phase mask can be separated from the other optical components, i.e. the design of the



Fig. 3. Experimental procedure for acquiring a Zernike phase contrast signal in scanning geometry in case of a ring type phase shifter (a) and an object consisting of a square (b). The sample is raster scanned through the focal plane of the lens at positions S_{ij} (c). After each movement, the bright-field cone image of the phase mask shows no contrast inside the dashed red lines when the focal spot is not on the object (d) and shows contrast when the object hits the focal spot (e). The final Zernike phase contrast signal for each scanning point p_{ij} is calculated by integrating the signal from the projection of the phase mask (f).

phase mask is not restricted provided that its full-field image can be masked in the detector plane. This is straightforward if a pixelated detector is used. This additional freedom in the design of the phase mask can be used to optimize the imaging properties of the SZPC, as will be shown in the following sections.

3. Artifacts and their origin in Zernike phase contrast imaging

Inherent to Zernike phase contrast equally in both full-field and scanning microscopy are imaging artifacts [11]. Typical are a halo and shade-off effect. Halos surround the edges of features and appear as bright or dark fringes, in case of positive and negative phase contrast, respectively. The shade-off describes a loss in contrast uniformity across larger uniform features where the contrast decreases gradually from the edges of the object to the center.

The underlying reason for these artifacts lies in the image formation process, where certain spatial frequencies are not properly transferred through the imaging system and as a result are suppressed in the final image [14]. Consider as an example the Fourier synthesis of a top-hat function, Fig. 4. When all spatial frequency components are present the top-hat has proper rectangular form. Suppression of Fourier components in the mid and lower frequency range will lead to a ringing appearance at its edges and a decreased value towards the center; in essence the higher spatial frequencies are overrepresented. This causes the artifact appearance in Zernike phase contrast images. The origin of this suppression of the lower spatial frequencies in full-field ZPC imaging lies in the dependence of the size of the scattering structures in the BFP of the OL because of the diffraction angles. The increase of the contrast is achieved by manipulating the phase of either of these light paths independently to the other. Unwanted spatial frequency suppression happens when the phase of one of the light paths is influenced when it was not

supposed to; like in the example when the scattered light gets erroneously phase shifted.



Fig. 4. Top hat function with all spatial frequencies present in its Fourier spectrum (green) and under-represented lower spatial frequencies (red).

As explained previously [6, 7, 8], in SZPC microscopy the roles of the source and the detector are interchanged. However, more importantly this reverses the roles of the phase shifter and the sample. The lateral size of the phase shifter in SZPC microscopy and the geometry of the setup define the spatial separation of the focal spot and the beam tails that can now be assimilated into the separation of the two independent light paths in the BFP of the OL in full-field Zernike phase contrast microscopy. The largest feature size of the sample leading to (correct) Zernike phase contrast is therefore defined by the lateral size of the phase shifter. Larger features representing lower spatial frequencies of the sample will lead to erroneous phase shifting and result to the shade-off effect and the typical halo surrounding feature edges.

The reason for both of these artifacts is closely linked and their reduction relies on the design of the phase shifter [12, 15]. It is evident that decreasing the size of the phase shifting features in the phase mask can reduce the effect of these artifacts. Further design parameters of the phase mask that influence artifact appearance are the spatial distribution of the phase shifting features as well as the relative area between phase shifting and non-phase shifting features (i.e. fill factor). We have explained in detail the general principle of contrast and artifact formation in the SZPC microscopy. In the following we show how the design of the phase mask affects to the Zernike phase contrast image and its artifacts.

4. Experimental setup

Figure 2 illustrates the measurement setup. As an objective lens a Fresnel zone plate (FZP) is used. The central stop (CS), which is placed in front of the FZP, and the order-sorting aperture (OSA), which is placed in between the FZP and the sample, are omitted from the figure for clarity. Both components are required to isolate the first-order focused X-rays from the FZP. The measurements were carried out at the Coherent Small-Angle X-ray Scattering (cSAXS) beamline of the Swiss Light Source (SLS), at the Paul Scherrer Institute in Villigen, Switzerland. X-rays with photon energy of 6.2 keV propagated through the phase mask, in which part of the X-rays were scattered. The phase mask was fabricated on a 20 μ m thick silicon membrane, by electron beam lithography (EBL) and reactive ion etching of silicon. The thickness of the phase shifting structures was 3.9 μ m, designed to produce a $\pi/2$ phase shift at the photon energy used. Several designs were fabricated to experimentally study the effect of the phase

mask design (see details in the next section). Downstream of the phase mask, the beam is focused onto the sample by an FZP with outermost zone width of 50 nm and diameter of 100 μ m made by electron beam lithography and gold electroplating [16]. These dimensions lead to a focal length of 25 mm. A Pilatus 2M pixel detector [17] with 172 μ m × 172 μ m pixel size is placed 7.5 meters downstream from the sample to collect the bright-field cone from the first diffraction order of the zone plate. Therefore, the phase mask is magnified onto the detector with a magnification factor of 300. Measurements were done by raster scanning the sample and recording the spatially resolved intensity distribution after each movement.

5. Design of the phase masks

All phase masks were based on square pillar or hole arrays in different arrangements. This is advantageous when using a pixel detector, as the square shaped pillar is imaged to a pixel or few pixels in the detector. Thus it allows the definition of the integration support to be as precise as possible. In other words, the square pillar arrays were designed to a grid defined by the demagnified detector pixel size. This defines the grid in the phase mask to $(172 \ \mu m \times 172 \ \mu m)/300 = 573 \ nm \times 573 \ nm$. The critical dimension (CD) of the pillars were varied from two to ten times of the width of the demagnified pixel, from now on referred as *p*. This leads to pillar sizes between 1.15 $\mu m \times 1.15 \ \mu m$ to 5.73 $\mu m \times 5.73 \ \mu m$. The fill factor, *f*, i.e. the fractional area covered by the pillars was varied from 1/4 to 1/16. Both regular and randomized pillar arrangements were used. Regular grids consisted of pillars on a square grid. Figure 5 illustrates the different geometries of the phase masks.



Fig. 5. Schematic illustration of a periodic phase mask with a fill factor of 1/4 (a), pseudorandom phase mask with a fill factor of 1/4 (b), and a phase ring (c). The meaning of the critical dimension (CD) is shown in figures (a) and (c).

A comprehensive study into effects of the phase mask on the computed Zernike phase contrast image was performed by varying three parameters of the phase mask, namely: CD of the pillars, fill factor in the pillar array, and the randomization of the pillars. These results were then compared to the previous design of the phase mask in SZPC microscopy, i.e. a simple ring structure [8]. The effect of the CD in the phase mask should have an influence that is directly related to the artifacts described above. By decreasing the feature size the beam tails of the scanning spot should reach further out, thus suppressing the artifacts. Increasing the fill factor was expected to increase the signal due to larger integration area of the Zernike phase contrast signal. The idea of randomization is that the beam tails in the illumination are expected to be smoother in case of randomized phase structures. This should reduce artifacts associated with sample feature sizes corresponding to the spatial frequencies on a regular grid. In the random phase masks the positions of the pillars were pseudo-randomly chosen only constraining the fill

factor of the whole phase mask.

The effect of the phase mask designs was evaluated by simulating the intensity distribution in the sample plane with different phase mask designs by using the experimental parameters described above. The simulations were done with scalar diffraction theory assuming an ideal lens in front of the phase mask. Ideally the intensity pattern should have a distinct focal spot produced by the lens and separated uniform background that acts as beam tails. Fig. 6 shows the simulated intensity distribution in case of a phase-shifting ring. As can be seen the focal spot is surrounded by fairly uniform beam tails.



Fig. 6. Simulated intensity distribution in the sample plane in log-scale by using a ring phase shifter with inner diameter of 50 μ m and width of 5 μ m (left). The scale bar is 5 μ m. The focal spot is the bright spot in the center of the figure. Radial line profile of the intensity distribution in log-scale (right).

Fig. 7 shows a similar simulation in the case of a phase mask consisting of square pillars on a square grid. The focal spot is surrounded by a regular grid of dots caused by different diffraction orders of the grid. By increasing the fill factor, the spots become sharper because the efficiency of certain diffraction orders is higher as the fill factor increases. As the CD is decreased the intensity in the beam tails is spread to a larger area.

Fig. 8 shows the simulation in the case of a square pillars on a random grid. Comparing Figs. 7 and 8, it is quite clear that the intensity in the beam tails is spread to an area with same size. However, in case of randomly arranged arrays, the beam tails are uniform without sharp spots compared to the regular grid arrangement. This makes the residual artifacts to appear smoother, and distinguishing them from the features of the specimen easier.

The total intensity in the focal spot from the total intensity in the sample plane varied from 25% to 10% depending on the phase mask design. In case of phase masks with largest fill factor, the total intensity in the focal spot was highest, and decreased by decreasing the fill factor. It should be noted that the fill factor is always below 0.5. The size of the pillar in the phase mask did not seem to have any effect on the intensity fraction in the focal spot.

To summarize the findings above, the simulations show that the CD of the phase mask determines how wide the spread of the beam tails is. In case of periodic pillar arrays, the beam tails consist of dots in the vicinity of the focal spot. With the randomized arrays the beam tails have uniform intensity without distinct features, which should reduce the artifacts. In addition, it seems that decreasing the fill factor further improves the uniformity of the beam tails, even though this effect is not very pronounced. Decreasing the fill factor has the negative effect of decreasing the amount of signal in the computed Zernike phase contrast image since the integration area is reduced with smaller fill factor. Based on these simulations the optimal phase mask consists of pillars with as small CD as possible arranged on a random grid. Furthermore, the choice of the fill factor should be a balance between artifact reduction and that it still provides enough signal within the integrated area. Figure 9 shows scanning electron microscopy



Fig. 7. Simulated intensity distribution in the sample plane in log-scale by using phase mask with pillars on a regular grid by varying the CD and the fill factor. The scale bar is $5 \ \mu m$.

(SEM) images of different types of phase masks.

6. Experimental results

Following the experimental procedure for acquiring the Zernike phase contrast signal (see also Fig. 3) the integration supports for integrating the Zernike phase contrast signal were defined manually for each phase mask. This was done by taking a bright-field cone image without a sample and with (bright-field) and without (flat field) the phase mask. The bright-field image of the phase mask was then divided by the flat field image revealing the positions of the imaged phase pillars in the detector. The centers of the individual pillars were defined manually and the size (the number of pixels for a single pillar) was chosen so that the defined dot in the integration support was smaller than or equal in size to the projection. The phase masks were not aligned to the detector pixel. However, the angle of the phase mask arrays were aligned to the detector pixel. It should be noted that the integration support for the bright-field cone images need to be defined only once for each phase mask, and for all the measurements with that particular phase mask, the same integration support can be used for calculating the Zernike phase contrast signal.

Images were recorded with 200 nm step size and 50 ms exposure time with a continuous scan. The total acquisition time was about 14 minutes/image. As a test sample, we used a Siemens star fabricated by EBL and nickel electroplating. The spokes of the Siemens star are empty and are surrounded by 100 nm thick nickel which results to $\approx 1\%$ absorption and 0.14 radian



Fig. 8. Simulated intensity distribution in the sample plane in log-scale by using phase shifter with square pillars on a random grid by varying the CD and the fill factor. The scale bar is 5 μ m.



Fig. 9. SEM-images of the different phase plate designs, periodic pillars (a), random pillars (b), and a ring (c). The scale bar in every image is 5 μ m.

phase shift. Figure 10 shows an example of Zernike phase contrast image of a test structure and two bright-field cone images of the phase mask related to two pixels as indicated in the Zernike phase contrast image formation. Horizontal stripes in Figs. 10(a) and 10(b) are due to the beamline optics. They do not affect the Zernike phase contrast images since the stripes remain constant during the scans. The empty spokes of the Siemens star appear darker in the ZPC image compared to the surrounding areas that are electroplated nickel. Due to the high sensitivity and low noise of the detector, the Siemens star also appears in the transmission image. However, the required normalization also reveals variations such as filling pattern of the storage ring.



Fig. 10. Zernike phase contrast image (c) using a phase mask consisting of a periodic pillar array on a square grid. The test sample is a Siemens star fabricated of electroplated nickel. Figures (a) and (b) present two Pilatus images that refer to pixels in computed Zernike phase contrast image indicated by the arrows. Outlined are three of the projected pillars from the phase mask, that are visible especially in (b). Transmission image of the Siemens star (d). The transmission image is not corrected for the variations of the incident intensity, thus showing the injection pattern of the synchrotron electron ring.

6.1. Effect of the critical dimension

Figure 11 shows the effect of the critical dimension (CD). All phase masks consist of square pillars in random arrangement with a fill factor of 1/9. Figure 11(c) with the largest CD shows typical features of the shade-off effect with intensity gradually increasing from the center to the edge of the feature. This artifact does not exist in Fig. 11(a) captured with the phase shifter with smallest CD. The effect can clearly be observed from the line profiles in Figure 11(d). With the largest CD, the structures are mainly visible because of the large contrast in the edges of the features.

6.2. Effect of the fill factor

Figure 12 shows the Ni Siemens star imaged with phase masks with different fill factors on a regular grid. The CD is 6p. As expected, the total signal within the integration support increases linearly with the fill factor as can be seen from the scale bars. The amount of signal could be an issue with detector producing more noise. However, photon-counting detectors have almost zero noise, and are not affected by this.

Figure 13 shows magnified images of the top left corner of the imaged Siemens star captured with the largest and the smallest fill factors. In the left image, we observe fringes surrounding the edges of both the dark and the bright spokes. These are not real features but artifacts. The artifacts are much less pronounced in the image taken with a phase mask with smaller fill factor due to the smaller scattering power to the first diffraction order. Increased scattering power to the first diffraction order creates clear features to the beam tails, that result in more pronounced



Fig. 11. Nickel Siemens star imaged with random pillar array phase mask with CD=2p (a), CD=6p (b), and CD=10p (c), and line profiles along the dotted red lines (d). In figure (d) black line, blue line, and red line are from figures (a), (b), and (c), respectively. The profiles are vertically shifted for better comparison.

halo formation in the image.

6.3. Effect of the randomization

Figure 14 represents the nickel Siemens star imaged with regular and random arrangements of the pillars in the phase mask. For both phase shifter arrays the CD is 6p and fill factor 1/4. It is evident that the randomized array produces a smoother halo compared to the periodic arrangement. The phase mask with periodic array produces sharp artifacts as can be seen in the cross-sections in Fig. 14(c).

From Figs. 11–14 it can be concluded that the pillar size has the biggest effect in reducing the shade-off and halos. This appears clearly in the large features of the images. The increase of the fill factor increases the halos especially in the case of periodic pillar arrays. The images produced by the randomized phase masks suffer least from the halo and shade-off effects provided that the pillar size is small enough.

Next we imaged the same Siemens star with higher resolution, decreasing the step size to 50 nm. Figure 15 shows a comparison of the Siemens star imaged with different types of phase masks. 100 nm lines and spaces indicated with an innermost ring of the Siemens star are resolved in every image. The figure captured with the ring clearly shows shade-off effect due to the large line width of the ring. The image captured with a periodic phase mask shows halo



Fig. 12. Nickel Siemens star imaged with periodic pillar phase masks with fill factor 1/16 (a), 1/9 (b), 1/4 (c), and line profiles along the dotted red line (d). In figure (d) black line, blue line, and red line are from figures (a), (b), and (c), respectively. The profiles are vertically shifted for better comparison.



Fig. 13. Top left corner of the Ni Siemens star imaged with phase masks with fill factor 1/4 (left) and fill factor 1/16 (right).

artifacts especially in the direction of the spokes. This can be seen as stripes in the image. The image captured with a randomized phase mask represents the cleanest image of the test structure in terms of reduced halo. It should be noted that the only difference in using a phase mask containing pillars ($-\pi/2$ phase shifter, negative ZPC) compared to one consisting of holes ($+\pi/2$ phase shifter, positive ZPC) is that the tone of the image is reversed. As expected, the tone of the phase mask showed no effect on the imaging artifacts.



Fig. 14. Nickel Siemens stars imaged with periodic pillar array (a), random pillar array (b), and line profiles along the dotted red lines (c). In figure (c) black line and blue line refer to images (a) and (b), respectively. The profiles are vertically shifted for better comparison.



Fig. 15. Nickel Siemens stars imaged by using periodic hole array (a), random hole array (b), a ring (c), and line profiles along the dotted red lines (d). In figure (d) black line, blue line, and red line are from figures (a), (b), and (c), respectively. The profiles are vertically shifted for better comparison.

Finally we imaged a slice of a *Chlamydomonas reinhardtii* cell, which is a species of green algae. *Chlamydomonas reinardtii* 137c Wt were obtained from the Chlamydomonas Genetic Center. Cells were cultured in TAP (Tris-acetate-phosphate) medium [18]. Cells in liquid TAP

medium were pelleted at 800g for 8 minutes. The pellet was fixed for 1 hour in M1 culture medium containing 2.5% glutaraldehyde, pH 7.2 at room temperature. After a rinse with distilled water the specimen was postfixed with 1% OsO_4 (in distilled water) at 4°C for 30 minutes. After washes with distilled water the sample was dehydrated using a graduated series of ethanol and infiltrated with Epon 812 with increasing concentrations of 33% (v/v) Epon in ethanol (2 h), 67% (v/v) in ethanol (2 h) and 100%. Polymerisation was in fresh, pure Epon for 72 h at 60°C. Sections of 300 nm thickness were cut with a diamond knife (ultra 35° Diatome) on a Reichert Ultracut S ultramicrotome and collected on formvar coated copped grids. The sections were stained with lead citrate and uranyl acetate [19].

The specimen was scanned using different phase mask designs, namely a phase ring, periodic, and random pillars with a fill factor of 1/4 and a CD of 6p. Scans were performed in a continuous fashion as described before, with 100 nm step size between consecutive acquisitions and an exposure time of 200 ms for each acquisition. The total acquisition time was 35 minutes/image. The dose D imparted on the specimen was estimated as $D = \mu N_0 \varepsilon / \rho$ [20], where $\mu = 1/342.3 \mu m^{-1}$ is the linear attenuation of epoxy resin [21], N₀ is the incident flux per unit area, ε is the photon energy, and $\rho = 1.14$ g/cm³ is the density of epoxy resin [22]. Taking into account the 7 m He (T \approx 97%), 13 μ m kapton window (T \approx 98%), and 8 μ m mica window (KAl₃Si₃O₁₂H₂F₂, T \approx 83%) dowstream of the sample this leads to a dose estimate of 6.1×10^6 Gy.

The processed images are shown in Figs. 16(a-c) for each type of phase mask, and clearly reveal the round shape of the cell section, where most of the contrast probably arises from the cell wall, and some internal structure due to different organelles. The image processed from the measurement with a ring type phase shifter, shown in Fig. 16(c), reveals the poorest contrast and strongest artifacts mainly due to shade-off caused by the large CD (line width) in the ring compared to Figs. 16(a) and 16(b), where the CD is 6p. For comparison we computed the differential phase contrast along the x and y directions [23], and then obtained a phase image as described in [24]. This analysis was done from the data acquired using the periodic phase mask. The resulting phase image, shown in Fig. 16(d), also reveals the same features of the specimen, but has less sensitivity compared to the Zernike phase contrast images. Outlined in Figs. 16 are two features that appear merged in the case of the periodic phase mask and separated in all the other cases.

7. Conclusion

In this paper we have investigated the effect of the design of the phase mask to the artifacts in SZPC microscopy. We have given a detailed description of the image formation based on Cowley's reciprocity theorem revealing that the roles of the phase shifter and the sample are interchanged. Therefore, SZPC imaging is in essence full-field imaging of the phase mask where the role of the sample is that of the phase plate. We have qualitatively explained the differences of the artifacts between different phase mask designs by simulating the intensity distributions in the sample plane. Experimental results clearly show the effect of the different phase shifter designs to the artifacts. The lateral dimensions of the phase shifting elements of the phase mask have the largest effect in the artifacts. Positioning the phase pillars randomly on the phase mask was shown to be advantageous compared to periodic arrangement for the artifact suppression. Smaller fill factor in the phase mask helps to suppress and smoothen the artifacts, however, this results in less signal in the computed image. Experiments using a classical ring structure also produces smooth halo, but in this case as well, the critical dimension (line width) would need to be minimized for better halo suppression. We showed an application example using a thin slice of a stained, polymerized chlamydomonas cell, where different organelles within the cell could be revealed. It should be noted that these findings are valid also for the



Fig. 16. Chlamydomonas cell imaged with periodic pillar array (a) randomized pillar array (b), and a ring (c), and integrated phase (d). Outlined areas in the red show isolated features that are not resolved in (a) imaged with periodic pillar array.

full-field Zernike phase contrast microscopy provided that the illumination can be coupled to the design of the phase plate.

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