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Fresnel coherent diffractive imaging tomography of whole cells in capillaries

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\textbf{Abstract}

X-ray tomography can be used to study the structure of whole cells in close to their native state. Ptychographic Fresnel coherent diffractive imaging (FCDI) holds particular promise for high-resolution tomographic imaging with quantitative phase sensitivity. To avoid the common missing wedge problem in tomography, cells can be mounted in thin glass capillaries that allow access to the full 180° angular field. However, soft x-rays, which are preferred for cellular imaging, interact strongly with capillaries, sometimes leading to violation of the usual assumptions for coherent diffractive imaging (CDI) and introducing artifacts (i.e., phase wrapping) in the reconstructed images. Here, we describe a
method of applying ptychographic FCDI to obtain quantitative x-ray phase images of whole eukaryotic cells mounted in capillaries. The approach eliminates phase-wrapping artifacts due to thick capillaries without the deterioration in image quality that occurs at shallow angles of incidence when using planar mounting schemes. This technique makes it possible to apply CDI tomography to the study of various specimens that can be supported in capillaries and is compatible with established methods of cryogenic preparation.

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Keywords: ptychography, soft x-ray microscopy, cellular imaging, Fresnel coherent diffractive imaging, capillary, tomography

1. Introduction

High-brilliance and highly coherent x-ray light sources have enabled x-ray coherent diffractive imaging (CDI) methods to be applied to three-dimensional (3D) reconstruction of samples with elemental sensitivity [1, 2] and at nanoscale resolution [3–5]. Recently, coherent x-ray diffractive imaging has been used to image the sub-cellular internal structure of biological cells in 3D with quantitative phase information [6–11].

A common method for obtaining 3D structure information is to mount the sample on a planar membrane and measure a series of projections. However, geometric constraints limit the range over which projections can be collected using a planar sample mount, creating a ‘missing wedge’ of data that can degrade the tomographic x-ray reconstruction [5, 12, 13]. The increasing absorption of x-rays through the sample at shallower incident angles can be even more of an issue for CDI since the reconstructed phase information may be degraded long before the measured intensity disappears. This problem can be avoided by mounting samples inside suitably prepared, hollow glass capillaries, which allow a full 360° rotation. Capillary features such as inner diameter and wall thickness can be tailored to match a particular sample using readily available equipment. Moreover, the capillaries themselves are relatively robust and can be easily modified to preserve biological samples in a hydrated state, for instance, within the vacuum environment of a soft x-ray microscope [8, 14].

Despite these advantages, when imaging capillary mounted samples using CDI, some complications remain. Unlike, for example, silicon nitride membranes which are often used for mounting cells and are generally thin and uniform enough such that the interaction of the wavefield due to the membrane may be neglected, the optical density of a capillary can easily be comparable to that of typical biological specimens. Thus, the interaction of the incident beam and diffracted beam with the capillary cannot be easily ignored. This is a major problem at very soft x-ray energies (e.g. <1 keV), which are of particular relevance for the imaging of biological specimens due to the high natural contrast between water and carbon available in the ‘water window’. However, even in the case of higher energy x-rays (e.g. 1–2 keV) the relative optical density of the capillary can be sufficiently high to produce phase wrapping artifacts, significantly degrading the quality of the resulting CDI reconstruction—as we demonstrate here.
In this paper, we demonstrate a method to improve the quality of reconstructed phase images of a specimen mounted in a capillary using ptychographic Fresnel coherent diffractive imaging (FCDI). The approach we describe introduces a modified step into the phase retrieval algorithm for ptychographic FCDI [3, 5, 15]. In particular, our method recovers the phase of the exit surface wave (ESW) by incorporating a model of the capillary transmission function in a modified frequency-space constraint applied to the iterative reconstruction algorithm. We show that this method eliminates the phase wrapping problems often associated with capillary mounted samples thereby increasing the quality of the corresponding 3D phase reconstruction.

2. Method

As a conceptual vehicle for understanding our approach, it is useful to briefly review the underlying principles of the FCDI [16–20] technique upon which this work is based. The FCDI technique uses a beam with well-characterized phase curvature, typically diverging from the focus of a Fresnel zone plate to illuminate the sample [16, 17]. A detector located in the far-field records an oversampled, continuous diffraction pattern. The structure of the sample is recovered using an iterative algorithm that enforces a priori information, including the known spatial extent of the sample. The use of FCDI has been shown to provide a unique solution [16] for the image reconstruction with fast convergence characteristics. Ptychography allows CDI techniques including FCDI to be applied to samples whose dimensions extend beyond the illumination area [2, 21–24], whilst providing distinctly improved image quality [21, 22, 24, 25]. Applying FCDI with ptychography [22] and more general schemes for introducing phase diversity in the illumination probe [23] has also been shown to yield greater phase sensitivity with less x-ray dose. Information redundancy is exploited in ptychography by recording diffraction data from multiple overlapping probe positions at the sample. The probe position reconstruction is then constrained in the overlap region image. FCDI ptychography has recently been implemented in 3D for both materials and biological samples [3, 5].

The following section describes the basic principle of a modified iterative FCDI technique [16, 17]. A priori information in the form of a model capillary transmission function is used to constrain the FCDI reconstruction of the specimen leading to improvement of the convergence in its reconstruction. The model is determined in the present case by simply measuring a region (or in other words, fitting a diffraction pattern corresponding to a region) of the capillary that does not contain any sample.

2.1. Implementation of modified modulus constraint

2.1.1. Creating a model capillary. On the lengthscales considered here we may approximate the capillary as an homogeneous and symmetric tube with inner and outer radii of \( r_1 \) and \( r_2 \), respectively. Hence, its projected thickness, \( t_c \), is given by

\[
t_c(r) = 2\left(\sqrt{r_2^2 - x^2} - \sqrt{r_1^2 - x^2}\right),
\]

where \( r = (x, y) \) is a vector defining a position in the transverse plane perpendicular to the propagation (projection) direction. Note that the long axis of the capillary is along the y-direction.
The transmission function of the model capillary, \( T_C(\mathbf{r}) \), can be expressed as

\[
T_C(\mathbf{r}) = \exp \left( -k\beta_C \mathbf{r} - i k \delta_C \mathbf{r} \right),
\]

where \( k = 2\pi/\lambda \) is wavenumber, \( i \) denotes the complex number, and \( \delta_C \) and \( \beta_C \) are respectively the real decrement from unity and the imaginary component of the capillary’s refractive index. The corresponding diffraction intensity from the capillary when illuminated by an incident monochromatic wavefield, which is characterized by \( \psi_o \), at a propagation distance \( z \), can be described as

\[
I_{\text{cap}}(\mathbf{r}, z) = \left| J_F \left\{ \psi_o T_C(\mathbf{r}) \right\} \right|^2.
\]

Here \( J_F \equiv AB/F \) and denotes the forward Fresnel transform with \( A \equiv -i \exp[ikz] \exp\left[ i\pi r^2/\lambda z \right]/\lambda z \) and \( B \equiv \exp\left[ i\pi r^2/\lambda z \right] \). \( J \) is the forward-Fourier transform operator. We note that \( \psi_o \) is reconstructed at the sample plane from a ‘whitefield’ image measured at the detector plane as described in [20, 26]. Here ‘whitefield’ refers to data acquired at the detector plane without either the sample or capillary in the beam.

Using optical microscopy images a coarse value for \( r_1 \) and \( r_2 \) was determined, whilst prior knowledge of the capillary composition allowed for an initial estimate of \( \delta_C \) and \( \beta_C \). Using these initial values, a model transmission function for the capillary was generated using (1.2) and corresponding diffraction intensities were calculated via (1.3). The four parameters (\( r_1, r_2, \delta_C \) and \( \beta_C \)) were then iteratively refined from the initial estimates to obtain the best fit to the experimentally measured capillary diffraction data.

### 2.1.2. Using a model capillary in a ptychography algorithm.

The actual sample used in the present work was composed of a fixed malaria infected red blood cell (RBC) placed inside of a capillary (C). The projection approximation in this case is considered to be valid provided \( z \gg r_2 \) [27]. The sample transmission function, \( T(\mathbf{r}, 0) \), is given by the product of the two component transmission functions of the cell, \( T_{\text{RBC}}(\mathbf{r}, 0) \), and capillary (C), \( T_C(\mathbf{r}, 0) \).

Hence, the sample ESW, \( \psi(\mathbf{r}, 0) \), can be expressed as

\[
\psi(\mathbf{r}, 0) = \psi_o T(\mathbf{r}, 0) = \psi_o T_C(\mathbf{r}, 0) T_{\text{RBC}}(\mathbf{r}, 0) = \psi_o T_C(\mathbf{r}, 0) + \psi_o T_C(\mathbf{r}, 0)[T_{\text{RBC}}(\mathbf{r}, 0) - 1].
\]

From (1.4), it can be seen that if the term \( \psi_A \) is treated as the incident wavefield modified by the interaction with the capillary, then \( \psi_B \) defines a new RBC-ESW in the sample plane. From (1.4), the corresponding sample ESW in the detector plane (located at a distance \( z \) from the sample) is

\[
\hat{\psi}(\mathbf{r}, z) = J_F \left\{ \psi_A(\mathbf{r}, 0) + \psi_B(\mathbf{r}, 0) \right\} = \hat{\psi}_A(\mathbf{r}, z) + \hat{\psi}_B(\mathbf{r}, z).
\]

Here \( \hat{\psi}_A(\mathbf{r}, 0) \) and \( \hat{\psi}_B(\mathbf{r}, 0) \) are, respectively, the modified incident wavefield and the ESW of the RBC propagated to the detector plane.

In ptychographic FCDI, the recovered sample ESW in the detector plane for a probe position indicated by index \( j \) can be expressed as
\[ \hat{\psi}_j (r, z) = J_F \left\{ \psi_o T_C (r - r_j, 0) + \psi_r T_C (r - r_j, 0) \right\} \times \left\{ T_{\text{RBC}} (r - r_j, 0) - 1 \right\} \]
\[ = J_F \left\{ \psi_{A_j} (r, z) + \psi_{B_j} (r, z) \right\} \]
\[ = \hat{\psi}_{A_j} (r, z) + \hat{\psi}_{B_j} (r, z). \]  

(1.6)

In ptychographic FCDI, the incident illumination at each scan position is subtracted from the current estimate for the far-field ESW in the detector plane before back-propagation to the sample plane. This ensures that the sample support constraint operates only on the updated sample transmission function [20, 22]. In the present work, we simply include the additional known information about the capillary transmission function by subtracting a modified whitefield given by \( \hat{\psi}_{A_j} (r, 0) \) rather than the standard \( \psi_o \) (e.g. the illumination with no capillary or sample in place). The following steps summarize our algorithm:

1. Reconstruct \( \psi_o \), as in the standard FCDI procedure, [16, 17, 26] then construct a modified whitefield, \( \hat{\psi}_{A_j} \), for each position in the ptychographic scan, given by

\[ \hat{\psi}_{A_j} (r, 0) = J_F \left\{ \psi_{A_j} (r, z) \right\} \]
\[ = J_F \left\{ \psi_o (r, 0) T_C (r - r_j, 0) \right\}, \]  

(1.7)

where \( r_j \) denotes the \( j \)th scan position and describes the lateral translation of the sample within respect to the incident beam.

2. Estimate the far-field ESW at the detector plane by using a random guess for the phase, \( \phi_{y_j} \), and the square root of the far-field intensity measured in the experiment, \( \sqrt{I_m} \):

\[ \hat{\psi}_j (r, z) = \sqrt{I_m} \exp \left( i \phi_{y_j} \right). \]  

(1.8)

3. Subtract the modified whitefield, \( \hat{\psi}_{A,m} \), from the result above and back-propagate it to the sample plane using

\[ \psi_{B_j} = J_F^{-1} \left[ \hat{\psi}_j (r, z) - \hat{\psi}_{A_j} (r, z) \right] \]
\[ = \left[ T_{\text{RBC}} (r - r_j, 0) - 1 \right] \psi_{A_j} (r, z), \]  

(1.9)

where \( J_F^{-1} \) denotes the inverse Fresnel transform.

4. Enforce the sample support constraint and propagate the result to the detector plane:

\[ \hat{\psi}_{B_j} (r, z) = J_F \left[ \pi_r \psi_{B,j} (r, 0) \right], \text{ where } \pi_r = \begin{cases} 1 & (r \in S) \\ 0 & (r \notin S) \end{cases}. \]  

(1.10)

The support area, \( S \), can be defined by using an approximation for \( \psi_{B,j} \) as described in [22, 28]. In this modified algorithm, \( S \) is firstly defined by a rectangle covering the RBC in the sample plane and then is iteratively updated using the shrinkwrap algorithm [28].
(5) Add the modified whitefield, $\hat{\psi}_A$, which was already determined as described above:

$$\hat{\psi}_j (r, z) = \left[ \hat{\psi}_{A_j} (r, z) + \hat{\psi}_{B_j} (r, z) \right]. \quad (1.11)$$

(6) Apply the modulus constraint to the result according to

$$\hat{\psi}_{j,\text{new}} (r, z) = \pi_m \hat{\psi}_j (r, z), \quad (1.12)$$

with $\pi_m = \sqrt{I_m/\hat{\psi}_{A_j} (r, z) + \hat{\psi}_{B_j} (r, z)}$.

(7) Steps (3–5) are then repeated for a predetermined number of iterations (i.e., $j = M$, if $M = 1$, it is updated at every iteration). The estimate of the transmission function of the RBC, $T_{RBC}(r, 0)$ is then updated by [21, 22]

$$T_{RBC}^{\ell+1}(r, 0) = T_{RBC}^{\ell}(r, 0) + \beta \sum_{j=1}^{J} U_j (r) \left[ \psi_{j, \text{new}}^{\ell+1} (r, 0) - \psi_{j, M}^{\ell} (r, 0) \right]. \quad (1.13)$$

where $U(r)$ is defined as

$$U (r) = \frac{\left| \psi_{A_j} (r - \mathbf{r}_j, 0) \right|}{\max \left\{ \psi_{A_j} (r, 0) \right\}} \times \frac{\psi_{A_j}^* (r, 0)}{\left| \psi_{A_j} (r, 0) \right|^2 + \alpha}. \quad (1.14)$$

The parameter $\beta$ in (1.13) is typically within the range of [0.9, 1]. The first term in (1.14) of $\left| \psi_{A_j} (r - \mathbf{r}_j, 0) \right|/\max \left\{ \psi_{A_j} (r, 0) \right\}$ is to ensure the transmission function is updated only in the region of the probe, $\psi_{A_j} (r - \mathbf{r}_j, 0)$. The second term is to normalize the probe function in the ESW, and $\alpha$ is a very small number, i.e., $\alpha \approx 10^{-4} \times \max \left\{ \left| \psi_{A_j} (r, 0) \right|^2 \right\}$ [21, 22], to prevent division by zero in (1.14).

Steps (3–7) are repeated until the square of the differences between the calculated and measured amplitudes is minimized [21, 22]. This approach can be readily applied to both ptychographic [22] and phase diverse methods [23] as well as to 3D reconstructions [3] using tomography techniques.

### 3. Experiment

The sample was a RBC infected with the malaria parasite, *Plasmodium falciparum*, at late trophozoite stage. The RBC was fixed in gluderaldehyde and injected into a drawn glass capillary before being allowed to dry at room temperature. The RBC adhered to the inner wall of the capillary. Details of the cell preparation have been published previously [5]. Figure 1 shows an optical microscope image of the RBC chosen for imaging inside the capillary.

Prior to injecting the RBC, the borosilicate glass capillary (Harvard Apparatus), was heated and drawn to thin its walls and reduce the inner diameter to about twice the diameter of the RBC. We note that thinner-walled capillaries can be produced using this method but their reduced mechanical stability complicates cell handling and mounting. Once injected, the sample (RBC and capillary) was mounted on a four-axis sample stage $(x, y, z, \theta)$. A rotation stage is on
top of the three-axis translation stage within the vacuum environment of the x-ray microscope [29].

The experiment was conducted at beamline 2-ID-B at the Advanced Photon Source, USA [30] which provides coherent x-rays in the range 630–2900 eV. An x-ray energy of 1810 eV, below the Si K-edge (1838 eV [31]), was used for this experiment because the borosilicate glass of the capillary has significant Si content. A 160 μm diameter Fresnel zone plate (FZP; XRadia) with a nominal outermost zone width of 30 nm and focal length of 7.01 mm at this energy was used to generate the curved illumination. The combination of a 40 μm diameter central stop at the FZP, supported by three spokes of width 15 μm, and a 20 μm diameter order sorting aperture was used to isolate the first order focus. A Princeton Instruments PI-MTE back-illuminated, Peltier cooled, charge coupled device with 2048 × 2048 pixels and 13.5 μm pixel size was positioned about 59.2 cm from the Fresnel zone plate focal point. A schematic of the experimental geometry is given in figure 2.
In order to characterize the capillary, data was collected from both an empty region of the capillary and a region containing RBC as discussed in the previous section. For the former, the capillary was placed 1589 $\mu$m downstream of the FZP focus in order to fit the whole capillary within the diverging beam. For the RBC data, the capillary was placed 282 $\mu$m downstream of the FZP focus. From the geometry, the estimated diameter of the illumination at the sample plane is 6.6 $\mu$m.

Ptychographic measurements that consisted of a 3 × 3 grid of probe positions with approximately 70% (4.6 $\mu$m) overlap [21] were performed with an exposure time (excluding CCD readout) of approximately 4000 s for a total x-ray fluence delivered to the capillary and RBC of $4 \times 10^{22}$ photons m$^{-2}$.

4. Results and discussions

4.1. Fitting the empty capillary diffraction pattern

Figure 3(a) shows the central region of the diffracted intensity from an empty part of the glass capillary where the diffraction pattern has been corrected [17] by subtracting the darkfield background and dividing the whitefield images. Here, ‘darkfield’ refers to images acquired without beam illumination. The high contrast vertical lines represent interference fringes from the capillary edges.

The model described by equations (1.1) and (1.2) assumes the capillary can be described as a symmetric tube with the inner and outer radii to be $r_1$, $r_2$ and homogenous composition characterized by its refractive index of which the decrement from unity of the real part is $\delta_C$ and imaginary part is $\beta_C$. A simulated diffraction pattern of the model capillary can be produced.
using (1.3) with the four parameters: \( r_1, r_2, \delta_C \) and \( \beta_C \). The initial values for these parameters were obtained from optical micrographs (figure 1) and by assuming a homogenous composition of 80.9\% SiO\(_2\), 12.9\% B\(_2\)O\(_3\), 4.4\% Na\(_2\)O, 1.8\% Al\(_2\)O\(_3\) for the capillary at the energy used in this experiment. The initial value of the capillary parameters were: \( r_1 \approx 6 \mu m \) and \( r_2 \approx 9 \mu m \), \( \delta_C = 1.24 \times 10^{-4} \) and \( \beta_C = 0.85 \times 10^{-5} \).

The values of \( r_1 \) and \( r_2 \) were refined by a least-squares fit of a simulated diffraction pattern from the model capillary to the experimentally measured diffraction pattern due to the real capillary. The values that were determined from fitting the experimental data in the present work were \( r_1 = 6.04 \pm 0.01 \mu m \); \( r_2 = 7.31 \pm 0.01 \mu m \); \( \delta_C = 1.53 \pm 10^{-4} \) and \( \delta_C = 1.01 \times 10^{-5} \). Figure 3(b) shows the diffraction pattern from the simulated glass capillary using those fitted values. The profiles along the dashed line for the measured and simulated capillaries are shown in figure 3(c) and demonstrate excellent correspondence between the two. This indicates that the values determined for the inner, outer radius, \( \delta_C \) and \( \beta_C \) values are accurate and that the model capillary is a good approximation of the real one.

### 4.2. Potential problems due to large phase shifts

Figures 4(a) and (b) show a schematic of two different projections of the RBC. In figure 4(a) both outer edges are outside the field of view and in figure 4(b) an edge of the capillary falls outside the field of view. Figures 4(c) and (d) show the corresponding projected phase shift profiles for the two cases in (a) and (b), in which the dashed-lines indicate the width of the positions of the illumination. From the known density and dimensions and from figures 4(c) and (d) it can be seen that the phase changes through the thickest region of the capillary (edge of the capillary, (c)) by approximately 12 rad and through walls (the middle of the capillary, (d)) by approximately 3.45 rad. The potential difficulty in identifying the specimen structure against this background of the capillary is clearly seen. Moreover, phase wrapping is expected to occur for phase shifts exceeding the range \([-\pi, \pi]\). Phase wrapping artifacts are indeed clearly seen in the reconstruction shown in figure 4(e). Artifacts are less visible in the case where the outer edges of the capillary are outside the illumination (figure 4(f)). These two ptychographic FCDI reconstructions were performed without using the modified algorithm described in this paper. Generally, phase unwrapping procedures are not particularly stable in the presence of noise [32]. Accordingly, attempts to apply phase unwrapping algorithms were not successful.

### 4.3. Using an accurate capillary model and tight support

Figures 4(g) and (h) show the reconstructions of the data acquired at the two projection angles generated using the capillary model and object support that is updated using the shrinkwrap algorithm. It can be seen that the quality of the reconstructed phase image is greatly improved in both cases. The structures visible in the reconstructed RBC image (shown in figure 4(h)) match those seen in the optical image (shown in figure 1) noting that the angle of the projection of the RBC in figure 4(h) is different from that for the optical image, shown in figure 1, by about 10–15°.

The improvement in the reconstruction shown in figure 4(h) is a result of using the known capillary model (with fitted parameters) so that only the RBC is reconstructed. Our approach avoids the need to reconstruct the whole sample (capillary and RBC) and therefore avoids phase-wrapping problems (as shown in figure 4(c)). Importantly, the object support can be defined close to the boundary of the RBC using the shrinkwrap method, rather than by the
extent of the illumination. With a tighter object support, reconstructions generally converge to a solution faster and with better quality, avoiding stagnation [28].

4.4. 3D IRBC reconstruction

The phase images reconstructed from 89 projections were aligned vertically using an auto-correlation procedure. They were aligned to a common rotation axis using an approach described in [33] before being combined to construct the 3D tomogram of the RBC using a filtered-back projection based tomography technique [34, 35]. A threshold was applied to remove the noise outside the tomogram. The threshold value was selected in such a way that
each projection of the threshold tomogram agrees with its corresponding 2D reconstructed phase. Using this approach, the resulting tomogram represents the quantitative 3D distribution of the decrement from the unity of the real part of the refractive index, $\delta(x, y, z)$, of the RBC.

The differential phase regions of the tomogram were segmented assuming that they represent the three major compartments expected at the late trophozoite stage [5, 36], i.e., the host RBC containing hemoglobin (Hb), the parasite cytoplasm with surrounding exomembrane system (Par), and hemozoin crystals in the parasite cytoplasm (Hz). To validate the initial segmentation, we used the quantitative phase information provided in the tomogram, and compared the measured densities of the three components to those previously reported in the literature. These values are presented in table 1, and show good agreement, supporting the initial segmentation of the tomogram. The small differences between calculated and previously reported values are most likely due to different sample preparation methods [5, 37]. Details of the method used for regarding the calculation of the density from the phase are presented in appendix A.

The 3D rendering of the RBC with the three compartments identified in table 1 is shown in figure 5. The rendering was performed by using Avizo software [11]. Each of three compartments was labelled and assigned by a different color. Figures 5(a) and (b) show two orthogonal views. Figure 5(c) shows a cross-section, through the RBC indicated by plane P in figure 5(a). The blue compartment (blue, transparent of 80%) indicates the host RBC, the green compartment

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**Figure 5.** (a), (b) 3D surface rendering of three recognized features in the reconstructed infected RBC at two orthogonal views; (c) a cross-section through the RBC at the position indicated by P plane in (a), the blue feature indicates the host RBC, the green feature indicates the parasite and its exomembrane system (Par), and the red features represent hemozoin (Hz) crystals within the parasite cytoplasm; (d) 3D rendering of the Hz as shown in (c).
Figure 5(c) shows only a 3D rendering of the Hz. By considering the geometry of the experiment, the effective size of a pixel in each recovered projection is found to be 14.7 nm and the diffraction limited resolution given by Abbe theory [38] is 40 nm. However, the spatial resolution that is actually achieved in CDI is limited by many factors [5, 39]. The aim of the present work was not to optimize resolution of the recovered 3D phase distribution of the RBC, but it can be estimated from the power spectrum density of a projection through the recovered volume [5] to be less than 100 nm.

5. Conclusion

We have shown an improvement in the quality of reconstructed phase images of a whole eukaryotic cell mounted in the capillary for data obtained by ptychographic FCDI. The improvement is obtained by incorporating an accurate model of the glass capillary into the illumination function that not only eliminates the need for reconstructing the capillary structure, which can cause phase wrapping problems due to its large phase shift, but also allows the use of a shrinkwrap algorithm to define a tighter object support for only the RBC. These two benefit lead to a significantly improved reconstruction. This method will find immediate applications in FCDI tomography of biological samples in close to their native state.

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Appendix A. Density calculation

Recall that \( \delta \) represents the real part of a refractive index of a compound voxel, which can be written as [40].
\[ \delta = \frac{r_e \lambda N_{\text{avo}}}{2\pi} \sum_{j=1}^{k} x_i f_{i j} \rho_i \left( \sum_{j=1}^{k} x_i A_i \right)^{-1} = \bar{\rho} - \frac{r_e \lambda N_{\text{avo}}}{2\pi} \sum_{j=1}^{k} x_i f_{i j} \left( \sum_{j=1}^{k} x_i A_i \right)^{-1}, \]

where \( r_e \) is the classical electron radius, \( N_{\text{avo}} \) is the Avogadro number, \( x_i, f_{i j} \) and \( \rho_i \) are the number of atoms, the real part of the atomic scattering factor and the mass density for an element type \( i \). \( k \) is the number of elements in the compound. The average density of the compound, \( \bar{\rho} \), can then be calculated as

\[
\bar{\rho} = \delta - \frac{r_e \lambda N_{\text{avo}}}{2\pi} \sum_{j=1}^{k} x_i f_{i j} \left( \sum_{j=1}^{k} x_i A_i \right)^{-1}.
\]

**Appendix B. 3D movie of the segmented RBC**

A 3D movie of the reconstructed RBC is available in the supplementary data, available from stacks.iop.org/NJP/16/093012/mmedia.

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