Digital holographic phase imaging with aberrations totally compensated

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Abstract: Digital holography is a well-accepted method for phase imaging. However, the phase of the object is always embedded in aberrations. Here, we present a digital holographic phase imaging with the aberrations fully compensated, including the high order aberrations. Instead of using pre-defined aberration models or 2D fitting, we used the simpler and more flexible 1D fitting. Although it is 1D fitting, data across the whole plane could be used. Theoretically, all types of aberrations can be compensated with this method. Experimental results show that the aberrations have been fully compensated and the pure object phase can be obtained for further studies.

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1. Introduction

Digital holographic phase imaging (DHPI) is widely used to quantitatively detect phase information with axial sensitivity in nanoscale [1,2]. With this technique, transparent samples such as cells can be visualized [1,3], without contrast agents (stains or fluorescence dyes) and in a completely noninvasive way. Thus, it has promising applications in the imaging of living cells, such as the monitoring of cell growth or the membrane fluctuations [4]. However, the phase of object is usually embedded in aberrations, which distort the object phase and prohibit accurate measurements unless they are fully compensated.

Methods for aberration compensation can be categorized as physical ones [5] and numerical ones [6–9]. Physical methods include, for example, using the exact same MO in both reference and object arm [10], using an telecentric imaging system [11–13], or taking extra object-free holograms [5,14]. Numerical methods, on the other hand, are typically implemented by quantifying and removing aberrations during the digital reconstruction process, and thus offer more flexibility.

In terms of numerical methods, compared to the first and second order aberrations (tilt and defocusing), higher order aberrations are much more difficult to compensate. Thus, some methods only compensate for the low order aberrations, leaving the higher order aberrations unresolved [15–17]. This will be not ideal if the system has high order aberrations.

One solution is two-dimensional (2D) surface fitting using pre-defined (high order aberrations included) aberration models [18,19], with the most acceptable technique being the 2D Zernike Polynomials fitting (ZPF) [18]. Many of these techniques, however, perform 2D fitting across the entire field of view (FOV) without considering the presence of object phase [18,19], leading to somewhat suboptimal results.

Comparing to 2D surface fitting, one-dimensional standard polynomials fitting (1DSPF) is more flexible, powerful and computation efficient, and theoretically, any type of line profile can be represented with 1D standard polynomial. Nevertheless, as pointed out by Colomb, T. et al. [20], traditional 1DSPF cannot characterize aberrations with cross terms, such as xy and x^2y.

In summary, although many efforts have been devoted to compensate the phase aberrations in DHPI systems, most of them request prior knowledge on the aberration models,
or are incapable of correcting high order aberrations especially those with cross terms, or negatively affected by the presence of object phase. DHPI system with aberrations totally compensated without the influence of object phase has not yet been demonstrated to our knowledge. Here we report a DHPI system that permits the wide-field phase image of object, with aberrations be totally compensated. Neither information about optical system nor any aberration models need to be known in advance. Experimental results show that the phase aberrations can indeed be fully compensated and the pure phase of object can be obtained for further analysis.

2. Principles

In this section, we first give a brief introduction on why aberrations always exist in the DHPI systems, and how they affect the phase measurement. Then, we give a detailed description on the aberration compensation procedure. Finally, its ability of aberration compensation is analyzed.

2.1 Interference and aberrations

In holographic systems, the interference pattern between object wave $O$ and reference wave $R$ can be described as

$$I = |O|^2 + |R|^2 + O'R + OR^*$$  \hspace{1cm} (1)

where $(\cdot)^*$ denotes the complex conjugation. The first two terms in Eq. (1) are zero terms and the others are cross terms. Either of the cross terms can be used to reconstruct the image of object. Here we use $OR^*$. The zero and twin terms can be removed with phase shifting [21] or off-axis techniques [11].

After extracting $OR^*$ from the holograms, it is numerically propagated to the image plane using angular spectrum method [22]. In the image plane, the object wave $O_i$ and reference wave $R_i$ can be defined as $O_i = A \exp(i\phi_O)$ and $R_i = B \exp(i\phi_R)$ where $A$ and $B$ are the amplitude while $\phi_O$ and $\phi_R$ are the phase of the object and reference wave, respectively. The object phase $\phi_O$ is the quantity of interest.

If there exists optical defects, the object wave and the reference wave will be altered as $O_{ia} = A' \exp(i\phi_{oa}) \exp(i\phi_{oa})$, $R_{ia} = B' \exp(i\phi_{ra}) \exp(i\phi_{ra})$ where $\phi_{oa}$ and $\phi_{ra}$ are the optical defects induced phase aberration. Thus the reconstructed wavefront becomes:

$$O_{ia}R_{ia}^* = A'B' \exp\left[i\left(\phi_o + \phi_u\right)\right]$$  \hspace{1cm} (2)

where $\phi_u$ is the total phase aberration and $\phi_u = \phi_{oa} - \phi_{ra} - \phi_{ra}$. It can be seen that the phase of object ($\phi_O$) is buried in $\phi_u$, which is consisted of the phase of reference wave ($\phi_R$) and the phase aberrations caused by the imperfections of system ($\phi_{oa}$ and $\phi_{ra}$).

The phase of object will be unaffected if the total phase aberration $\phi_u$ is constant, which is impractical in real case. Because even if the optical system has no defects, which means $\phi_{oa}$ and $\phi_{ra}$ are all constant, the object phase is still affected by $\phi_R$. For example, in the widely used off-axis holographic system, the angle between the reference wave and the object wave induces tilt into $\phi_R$, which causes tilt aberration. Moreover, if a MO is used to magnify the object, quadratic phase is introduced into $\phi_{oa}$, causing defocusing aberration. Aberrations will be more sophisticated if the system is imperfect, i.e. it has optical defects.

It can be seen that in real case, it is almost unavoidable that the object phase is affected by various kinds of aberrations. Therefore, for an accurate measurement of the object phase, the total phase aberration $\phi_u$ must be fully quantified and compensated.
2.2 Fitting procedure and aberration compensation ability

It is easy to find out the amount of aberrations in the background and then compensate them. However, the work is difficult for the object areas because in these areas the object phase are messed up with aberrations. Thus, we should use the aberration data in the background area to estimate that in the object area. When estimating aberrations, the object areas should be removed manually [20] or automatically with background detection methods [12], to get rid of their negative influence. Therefore, here, after unwrapping the reconstructed phase image [23], the background without object area is extracted to obtain a 2D phase distribution \( \phi \) that will be used to quantify the phase aberrations. If aberrations have been fully compensated, the background will be flat or its standard deviation (STD) will be very small [20].

Fig. 1. Procedure for aberration compensation. STD, standard deviation; \( \phi_{re} \), residual phase aberration; \( \phi_{q} \), quantified phase aberration; 1DSPF, one-dimensional standard polynomials fitting.

The process of aberration compensation is given in Fig. 1. Here we use \( \phi_{re} \) and \( \phi_{q} \) to represent the “residual phase aberration” and the “quantified phase aberration”, respectively. Initially, \( \phi_{re} \) equals to \( \phi \). We calculate the STD of \( \phi_{re} \). If STD is larger than a threshold value \( t \), we find the direction \( u \) along which the STD is maximum using grid search. The grid search is realized by calculating the STDs of line profiles separated by 2°. After this, extract the profiles along direction \( u \) and its normal direction \( v \). Then using 1DSPF, two extracted profiles are fitted to functions \( f_u \) and \( f_v \), respectively, as:

\[
\begin{align*}
    f_u &= a_0 + a_1 u + ... + a_n u^n \\
    f_v &= b_0 + b_1 v + ... + b_n v^n
\end{align*}
\] (3)
where \(a_n\) and \(b_n\) \((n = 0, 1, 2, 3\ldots)\) are the polynomial coefficients of order \(n\). The order of polynomial function \(n\) can be set as needed. Here, we limit the correction to fourth order \((n = 4)\) as it already includes nearly all of the ordinary aberrations encountered in an optical system.

Assume the angle between \(uv\) and the principle axes \(xy\) is \(\theta\). According to the coordinates transform, the quantified 2D phase aberration \((\phi_q)\) can be calculated as:

\[
\phi_q = \sum_{k=0}^{4} \sum_{l=0}^{4} P_{k,l} x^k y^l, k + l \leq 4
\]

where \(x, y\) are the coordinates of the entire FOV (including the object areas), \(P_{k,l}\) is the coefficient of aberration \(x^k y^l\). The relationship between aberration coefficients \(P_{k,l}\) and polynomial coefficients \((a_n\) and \(b_n)\) is given in Table 1.

**Table 1. Relationship between the aberration coefficients \(P_{k,l}\) and the polynomial coefficients \((a_n\) and \(b_n)\)**

| Coefficients | Aberrations | Description | Expression |
|--------------|-------------|-------------|------------|
| \(P_{0,0}\)  | 1           | piston      | \(\max(a_n, b_n)\) |
| \(P_{1,0}\)  | \(x\)      | \(x\)-tilt  | \(a_1 \cos \theta - b_1 \sin \theta\) |
| \(P_{0,1}\)  | \(y\)      | \(y\)-tilt  | \(a_1 \sin \theta + b_1 \cos \theta\) |
| \(P_{2,0}\)  | \(x^2\)    | \(x\)-cylinder | \(a_2 \cos^2 \theta + b_2 \sin \theta\) |
| \(P_{0,2}\)  | \(y^2\)    | \(y\)-cylinder | \(a_2 \sin^2 \theta + b_2 \cos^2 \theta\) |
| \(P_{1,1}\)  | \(xy\)     | cross-cylinder | \(2(a_2 - b_1) \sin \theta \cos \theta\) |
| \(P_{3,0}\)  | \(x^3\)    | \(x\)-arrow  | \(a_3 \cos^3 \theta - b_3 \sin \theta\) |
| \(P_{1,3}\)  | \(y^3\)    | \(y\)-arrow  | \(a_3 \sin^3 \theta + b_3 \cos^3 \theta\) |
| \(P_{1,2}\)  | \(xy^2\)   | \(2(a_2 \cos \theta \sin \theta + b_2 \cos^2 \theta)\) |
| \(P_{2,1}\)  | \(x^2y\)   | \(3(a_2 \sin \theta \cos \theta + b_2 \sin \theta \cos \theta)\) |
| \(P_{4,0}\)  | \(x^4\)    | \(a_4 \cos^4 \theta + b_4 \sin \theta\) |
| \(P_{0,4}\)  | \(y^4\)    | \(a_4 \sin^2 \theta + b_4 \cos^4 \theta\) |
| \(P_{2,2}\)  | \(x^2y^2\) | \(6 \sin \theta \cos^2 \theta (a_4 + b_4)\) |
| \(P_{4,1}\)  | \(x^4\)    | \(4(a_4 \cos^4 \theta \sin \theta - b_4 \sin \theta \cos \theta)\) |
| \(P_{1,3}\)  | \(xy^3\)   | \(4(a_4 \sin^3 \theta \cos \theta - b_4 \sin \theta \cos^3 \theta)\) |

In Table 1, it should be noted that, the polynomial coefficient \(a_n\) only influences the coefficients \(P_{k,l}\) of aberrations \(x^k y^l\) where \(k + l = n\). For example, \(a_2\) only influences the coefficients of aberrations \(x^2, y^2\) and \(xy\) while the other aberrations are not related. On the other hand, to quantify coefficient \(P_{k,l}\), polynomial coefficients \(a_n\) and \(b_n\) where \(n = k + l\) are both needed. For example, to obtain the coefficient of aberration \(xy^2\), polynomial coefficients \(a_3\) and \(b_3\) are both needed.

It can be seen that we use the fitted polynomial coefficients, \(a_n\) and \(b_n\), to calculate the aberration coefficients \(P_{k,l}\) according to Table 1. Then use them to calculate the quantified 2D phase aberration \((\phi_q)\) according to Eq. (4). Using Eq. (4), aberrations across the whole plane, including that in the object areas, are obtained and compensated as \(O\_\text{ia} R\_\text{ia} * \exp(-i\phi_q)\). After this, the residual phase aberration \(\phi_re\) changes to \(\phi_re = \phi_re - \phi_q\). The \(\phi_re\) will be fitted again and the whole procedure will be repeated, until its STD is smaller than the threshold \(t\).

From Eq. (4), we can see that all kinds of aberration (limited to \(n \leq 4\)) have been quantified including those high order aberrations and those with cross terms. Higher order aberrations can also be included with larger \(n\). As we mentioned before, the order of
polynomial function $n$ can be set as needed. However, one should note that, if the highest order aberration of is $x^K y^L$, $n$ should satisfy $n \geq K + L$. Thus, $n$ should be set according to the highest order of aberrations. Usually, the fourth order ($n = 4$) is good enough.

3. Experiments

Experiments were implemented to test the ability of aberration compensation. Figure 2 shows the schematic setup based on a Mach-Zehnder interferometer. Both reflection geometry (Fig. 2 (a)) and transmission geometry (Fig. 2 (b)) were used. For both systems, a 473 nm laser was spatially filtered and expanded, with a neutral density filter to adjust its intensity. A polarizing beam splitter (PBS) divided the light into two beams, one reflected by a spatial light modulator (SLM, Holoeye, Pluto-VIS) worked as the reference wave, while the other one reflected by (or transmitted through) the sample worked as the object wave. A half-wave plate was used to adjust the relative intensity of two waves. The two waves were combined together by BS2 and a polarizer P was used to get the same polarization direction. The holograms were recorded by a complementary metal oxide semiconductor (CMOS) camera (Thorlabs, DCC3260M).

For reflection imaging, lens L1 and an infinitely corrected MO (Olympus, UPLSAPO 40X/0.95) were combined for wide-field illumination. Light reflected by the object was collected by same the MO. For the transmission imaging, lens L1 and MO1 (Daheng Optics, GCO-2105 40X/0.65) were combined for wide-field illumination while another infinitely corrected MO2 (Olympus, MPLFLN 20X/0.45) and lens L2 were combined for wide-field imaging.

![Fig. 2. Schematic of the DHPI system, (a) for reflection imaging and (b) for transmission imaging. NF, neutral density filter; BE, beam expander with spatial filter; λ/2, half-wave plate; BS, beam splitter; SLM, spatial light modulator; L, lens; P, polarizer; MO, microscope objective; CMOS, complementary metal oxide semiconductor. The focal length of lens L1 and L2 are 300 mm.](image)

The interference configuration can be either on-axis or off-axis depending on the angle between the reference wave and the object wave. Here, the SLM in reference arm was used for phase shifting to separate the desired wavefront $OR^x$. One can also use the off-axis technique without the need of phase shifting.

Meanwhile, to demonstrate the strong ability of aberration compensation, the SLM was also used to introduce aberrations on purpose and the reference wave was tilted to induce large amount of tilt aberration. Thus, the total aberrations included the tilt of reference wave, the quadratic aberration induced by MO, the aberrations intentionally introduced by SLM and the other aberrations caused by the defects of optical system.

3.1 Reflection imaging

As a proof of principle, we first imaged a positive 1951 USAF target (Daheng Optics, GCG-020601) with the reflection system. Figure 3 shows the reconstructed intensity image (a), the wrapped (b) and unwrapped (c) phase image. From the intensity image, an Arabic number “3” is clearly visible. However, from the phase image, it is hard to observe any object even after
phase unwrapping. That is because the phase of object was embedded in large amount of aberrations. To obtain a pure phase image of object, aberrations have to be removed.

Fig. 3. Reconstructed intensity (a) and phase distribution before (b) and after (c) unwrapping. Only regions of interest (ROI) are shown. The color bars indicate the phase in radians. Scale bar: 20 μm.

We used the procedure described in Section 2.2 to quantify and remove phase aberrations present in Fig. 3(c). The object area was manually excluded based on the intensity image in Fig. 3(a). We set the STD threshold \( t = 0.46 \) and the algorithm terminated after 10 iterations. The residual aberrations \( \phi_r \) (left) and the quantified phase aberrations \( \phi_q \) (right) for iteration 1 to 7 are shown in Fig. 4. The residual aberrations are presented with object areas un-removed and in wrapped status for clear observation.

![Residual phase distribution and quantified aberrations](image)

Fig. 4. Residual phase distribution (left) and quantified aberration \( \phi_q \) (right) in each iteration. From (a) to (g), the times of iterations are 1, 2, 3, 4, 5, 6 and 7, respectively. Yellow arrows indicate the uv directions along which the fitting was executed. The residual phase distribution is showed in wrapped status. Color bar for wrapped phase: from black to white the values are \(-\pi\) to \(\pi\). The other color bars indicate the phase in radians. Scale bar: 20 μm.

In Fig. 4, we can see that the aberrations have been gradually reduced with increasing number of iterations. For example, there are \(-5\) periods of wrapped aberration \((-\pi\) to \(\pi\)) in Fig. 4(d), whereas in Fig. 4(g), it is reduced to less than 1 period. Meanwhile from the color
bars of the right figure, it can be seen that the quantified aberration became smaller and smaller with iteration, also implying the decrease of aberration. For more accurate analysis, the STDs of residual phase aberration (without the object area) are given in Table 2. Generally speaking, in Table 2 the STD reduces with iterations until it reaches the threshold. All of these prove that the aberrations have been gradually reduced.

We note that, in Table 2, after the first iteration, the STD reduced dramatically from 226.39 to 7.95. This is because most part of aberrations is the low order aberrations (tilt and defocusing) that are easy to compensate. However, high order aberrations are more sophisticated and hard to compensate. Thus more iterations are required for full compensation.

Table 2. Fitting directions and STDs of residual aberration in each iteration

| Iteration | 0   | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  |
|-----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Directions (degrees) | 4   | 48  | 6   | 48  | 0   | 48  | 4   | 48  | 4   | 4   | 44  |
| STD       | 226.39 | 7.95 | 8.56 | 4.37 | 2.46 | 1.19 | 0.65 | 0.56 | 0.47 | 0.49 | 0.45 |

Table 2 also gives the directions along which the fittings were executed. These directions are also indicated with yellow arrows in Fig. 4. During iteration, the method automatically searches the whole 2D plane to find the direction with the maximum aberration. Theoretically, data across the whole 2D plane could be used. Here, in Table 2, we can see that directions mainly focused on five directions because the aberrations are dominant on these directions.

Figure 5(a) shows the phase distribution, in which only the low order aberrations (tilt and defocusing) have been compensated. In this case, the object phase is distorted by the uncompensated high order aberrations such as astigmatism and coma. The STD on the object area (number “3”) is 1.45. Although comparing to low order aberrations, the amount of high order aberrations is small, they still need to be compensated. Figure 5(b) shows the object phase after compensating the total aberrations. We note that the STD of the background of Fig. 5(b) has been reduced from 226.39 to 0.45, while that of Fig. 5(a) only has been reduced to 5.59. In Fig. 5(b), the phase of object can be clearly observed without disturbance and its STD is 0.34, which is comparable with that of noise. That means the constant phase on number “3” is obtained without aberration distortion, proving that aberrations have been fully compensated with our method.

3.2 Transmission imaging

Here we show that our method can also be used to correct for the aberrations in the phase imaging of biological samples. We imaged both murine myoblasts C2C12 cells and human breast cancer MCF-7 cells with the transmission system. These cells were kindly provided by Stem Cell Bank, Chinese Academy of Science. They were cultured in Dulbecco's modified Eagle's medium (DMEM, high glucose, Gibco) supplemented with 10% fetal bovine serum (FBS, Hyclone) at 37°C and 5% CO₂. Once reaching 90% confluence the cells were exposed to trypsin (0.25% w/v) and used for experiments. The cells were re-suspended in DMEM
medium to final density of $5 \times 10^4$ cells/mL, and seeded on cover slips. After 6h incubation at 37°C, the samples were fixed in 4% paraformaldehyde for 4h at 4°C.

Fig. 6. Intensity (a) and phase images (b, c, d) of C2C12 cells. The phase before and after aberration compensation are given in (b) and (c), respectively. The area near the yellow arrow in (c) is enlarged and inversed in (d) with pseudo three-dimensional display. The brightness of intensity image has been increased 15% for observation. The color bars indicate the phase in radians. Scale bar: 20 μm.

The intensity image of the C2C12 cells is shown in Fig. 6(a). Since these cells are mostly transparent, they are hardly visible in the intensity image as indicated by the yellow arrow (except when out-of-focus as marked by the red arrow). Phase imaging is an ideal way to visualize such samples due to index differences between the cells and their surroundings. However, because of the aberrations, the phase of the cells is difficult to observe as in Fig. 6(b).

We then ran our algorithm for 2 iterations, and the compensation result is shown in Fig. 6(d). We observe that after compensation, the cell structures are much more visible with a spindle-like morphology. Their structures can be clearly observed including the nucleus and their inner structure. The flat background suggests that the aberrations have been compensated. Quantitatively, the STD of the residual phase was reduced from 298.85 to 0.36.

Figure 7 shows the phase image of MCF-7 cells after aberration compensation with conventional 2D ZPF (a) and our method (b). The STDs of residual aberrations on the background of Fig. 7(a) and Fig. 7(b) are 0.36 and 0.33, respectively. The area near the red arrow in Fig. 7(b) is enlarged and showed in Fig. 7(c) with pseudo three-dimensional display. Figure 7(d) shows the reversed phase data of (a) and (b) along the yellow line profile in Fig. 7(a).

In Fig. 7(a) and (b), we observe that both methods can remove the aberration and the MCF-7 cells show a typical epithelial like shape. However, the 2D ZPF presents a small amount white region around each cell (indicated by green arrows in Fig. 7(a)). This is more obvious in Fig. 7(d), in which as the green arrows indicated, there are singularities around
cells. It is possibly caused by the negative effect of object phase, so the quantified phase aberration is not entirely accurate. Such artifact is not present with our method.

![Fig. 7. Phase distribution of MCF-7 cells after aberration compensation with 2D ZPF (a) and 1DSPF (b). The area near the red arrow in (b) is enlarged, reversed and showed in (c) with pseudo three-dimensional display. Reversed phase distributions of (a) and (b), along the yellow line profile in (a), are presented in (d) with red dotted line and blue solid line, respectively. The color bars indicate the phase in radians. Scale bar: 20 μm.](image)

From the experiments in Fig. 6 and Fig. 7, it is can be seen that our method can compensate the total aberrations presented in the DHPI of cells. The nucleus and inner structures can be clearly observed for further studies.

4. Summary

In conclusion, we use 1DSPF to compensate the aberrations existed in DHPI system. The 1DSPF is adaptively implemented along direction with the maximum aberration and its vertical direction. Then using the relationship between the 1DSPF coefficients and the coefficients of aberrations, the quantified 2D aberration is obtained and removed. The residual aberrations will be fitted again. This process is executed iteratively until the residual aberration is small enough. Additionally, the influence of object phase can be removed. Experimental results show that this method can compensate the total phase aberration, including those high order aberrations with cross terms. Neither pre-defined aberration models nor priori information about optical system are required.

This method solves the tough and unavoidable phase aberration problem existed in DHPI systems. It is powerful and aberration can indeed be fully and effectively removed, advancing the development and applications of DHPI.

We should point out that here we only demonstrated the aberration compensation on a single plane. In the case of 3D objects where phase aberrations across different object layers are different, for aberrations on each object layer, they can be individually compensated with this method. Moreover, this method requires that samples should have some flat background.

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Disclosures

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