Optics & Laser Technology 57 (2014) 169–174

Contents lists available at ScienceDirect

Optics & Laser Technology

journal homepage: www.elsevier.com/locate/optlastec

Automatic compensation of phase aberrations in digital holographic microscopy for living cells investigation by using spectral energy analysis

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ARTICLE INFO

Article history:
Received 18 June 2013
Received in revised form
9 October 2013
Accepted 12 October 2013
Available online 2 November 2013

Keywords:
Digital holographic microscopy
Phase aberration compensation
Spectral energy analysis

ABSTRACT

Phase aberration compensation is crucial for quantitative phase-contrast imaging in digital holographic microscopy. In this paper, an automatic compensation method is proposed for living cells investigation in digital holographic microscopy. The phase aberrations are extracted and corrected automatically with a single hologram by using spectral energy analysis. Zernike polynomials are adopted to model the phase aberrations. The polynomial coefficients related to the amount of phase aberrations are calculated in a nonlinear optimization procedure, in which a spectral energy metric that places more weight on low-frequency components is maximized. The effectiveness of the proposed method is demonstrated with experimental result of mouse osteoblastic living cells.

1. Introduction

Digital holographic microscopy is a promising technique in the study of microscopic objects. It permits non-invasive, label-free and quantitative phase-contrast imaging, which makes it highly suitable for the investigation of biological living cells [1–8]. However, the optical components used in the imaging system, especially the microscope objective, also induce undesired phase aberrations. These phase aberrations affect the accurate retrieval of specimen phase information, and must be compensated before quantitative analysis.

Several methods have been utilized to compensate phase aberrations in digital holographic microscopy. A physical compensation method is realized by using a symmetric setup that employs two microscope objectives in both interferometric branches [2]. This approach relies on precise alignment to counteract the phase curvature, thus complicating the experimental work. Another strategy is similar to the double exposure approach used in holographic interferometry, in which the phase aberrations are recorded with a reference hologram captured in the absence of the specimen and then subtracted from the object phase image [9]. This method is accurate when the imaging system is stable enough, but it needs to capture an additional hologram which could be unreliable for long-term observation or complicated imaging system. Numerical compensation methods are proposed as well, taking advantage of digital lateral shear [10], reference conjugated hologram [11], wavefront folding [12], etc. Compared with these approaches, it is more flexible to introduce a digital phase mask in the recording plane or the image plane to correct phase aberrations. Cuche et al. constructed the digital phase mask by adjusting a set of parameters to achieve aberration correction, but the adjustment is based on a priori knowledge of the optical setup [13]. Colomb et al. defined the phase masks with standard or Zernike polynomials and then obtained the polynomial coefficients by using phase unwrapping and least square fitting methods [14,15]. This approach is remarkably effective, but the phase unwrapping and fitting procedures should be performed in the area where the specimen information is known to be flat. The selection of this area requires manual intervention in most cases, which restricts the automaticity of aberration compensation. Miccio et al. modified this strategy by considering the specimen is thin enough, so that the phase unwrapping and least square fitting procedures could be implemented in the whole region of interest [16]. However, the availability of this method is limited when the phase contribution of the specimen is comparable to the phase aberrations.

In this paper, we propose a numerical method to correct phase aberrations in living cells investigation by digital holographic microscopy. It differs from previous methods in the extraction of phase aberrations. A spectral energy metric that puts more weight on low-frequency components is defined based on the

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http://dx.doi.org/10.1016/j.optlastec.2013.10.014

understanding of the spectral energy variation caused by the phase aberrations. A digital phase mask defined with Zernike polynomials is used to simulate and correct the phase aberrations. The polynomial coefficients that describe the degrees of phase aberrations are generated by maximizing the spectral energy metric in a nonlinear optimization procedure. The compensation is a totally automatic process and works with a single hologram. The capability of the proposed method is demonstrated with experimental result of mouse osteoblastic living cells.

2. Method

In digital holographic microscopy, a digital camera is adopted to capture the hologram formed by the interference of the object wave and the reference wave. The object wavefront is reconstructed from the digital hologram with numerical methods. After the reconstruction procedure, we could obtain the discrete complex-amplitude distribution of the object wave in the image plane, which could be written as $\psi(x,y)$. Subsequently, the amplitude image $A(x,y)$ and the phase image $\Phi(x,y)$ are calculated by

$$A(x,y)=|\psi(x,y)|=\sqrt{\mathrm{Re}\{\psi(x,y)\}^2+\mathrm{Im}\{\psi(x,y)\}^2}, \tag{1}$$

$$\Phi(x,y)=\arctan\frac{\mathrm{Im}\{\psi(x,y)\}}{\mathrm{Re}\{\psi(x,y)\}}, \tag{2}$$

where arctan denotes the four-quadrant arctangent operator, Re{} and Im{} represent the real and imaginary parts, respectively. As the phase image $\Phi(x,y)$ reflects the optical path difference, it could be used to analyze the morphological features of the specimen. Unfortunately, the phase image is degraded due to the phase aberrations induced by the imaging system. Taking the phase aberrations into account, the reconstructed complex-amplitude image is expressed as

$$\psi(x,y)=\psi_o(x,y)\exp[i\varphi_e(x,y)], \tag{3}$$

where $\varphi_e(x,y)$ denotes the phase aberrations, $\psi_o(x,y)$ denotes the ideal image without phase errors. The phase aberrations could be simulated by a digital phase mask $\varphi(x,y)$ defined with Zernike polynomials

$$\varphi_e(x,y)\approx\varphi(x,y)=\sum_n a_n Z_n(x,y), \tag{4}$$

where $Z_n(x,y)$ denotes the Cartesian form of the n-order Zernike polynomial, a_n denotes its coefficient. The aberration-corrected image $\psi_c(x,y)$ is hereby obtained by

$$\psi_c(x,y)=\psi(x,y)\exp[-i\varphi(x,y)] \tag{5}$$

Since the phase aberrations are represented by the polynomial terms, the effectiveness of this compensation procedure depends on the generation of adequate polynomial coefficients.

For biological living cells investigation, digital holographic microscopy usually works in transmission mode as the specimen exhibits weak light absorption. Quantitative phase-contrast imaging is caused by the different refractive indices of the cellular structures and the liquid culture medium. The phase profiles of the living cells are generally smooth, while the culture medium provides a flat phase background. Therefore, the spectral energy of the undistorted image $\psi_o(x,y)$ is mainly located at low-frequency components, especially at zero-frequency component. In contrast, much of the spectral energy of the distorted image $\psi(x,y)$ moves to high-frequency area, which is mainly due to the spectral shift caused by the phase tilt and the spectral expansion related to the phase curvature. After accurate aberration compensation, the spectral energy distribution of $\psi_c(x,y)$ should be similar to that of $\psi_o(x,y)$. This recovery could be evaluated by means of Fourier spectral analysis. The two-dimensional discrete Fourier transform (DFT) of $\psi_c(x,y)$ is written as

$$\begin{aligned}F(\xi,\eta)&=DFT[\psi_c(x,y)]\\&=\sum_{x=0}^{N-1}\sum_{y=0}^{N-1}\psi(x,y)\exp[-i\varphi(x,y)]\exp\left[-\frac{2\pi i}{N}(x\xi+y\eta)\right],\end{aligned} \tag{6}$$

where N is an even integer denoting the image size, ξ and η are discrete frequency coordinates from 0 to $N-1$. In order to investigate the spectral energy features, we hereby define a weighted spectral energy metric

$$M=\sum_{\xi=0}^{N-1}\sum_{\eta=0}^{N-1}w(\xi,\eta)|F(\xi,\eta)|^2, \tag{7}$$

where $w(\xi,\eta)$ is the weight function to evaluate different frequency components

$$w(\xi,\eta)=\frac{1}{1+\sqrt{\xi'^2+\eta'^2}}, \tag{8}$$

where

$$\xi'=\begin{cases}\xi, & if\ 0\le\xi<\frac{N}{2}\\ \xi-N, & if\ \frac{N}{2}\le\xi<N\end{cases},\quad \eta'=\begin{cases}\eta, & if\ 0\le\eta<\frac{N}{2}\\ \eta-N, & if\ \frac{N}{2}\le\eta<N\end{cases} \tag{9}$$

The piecewise definition of $w(\xi,\eta)$ is on symmetry reasons. Obviously, $w(\xi,\eta)$ places more weight on low-frequency components with a maximum of $w(0,0)=1$. It could be inferred that the spectral energy metric M is maximized when the phase aberrations are well compensated. The aberration correction process is therefore converted to an optimization problem. The purpose is to find a group of Zernike polynomial coefficients a_n that could maximize the spectral energy metric M. This is an unconstrained nonlinear optimization problem, which could be solved by using trust-region Newton algorithm [17,18]. The implementation of this algorithm requires the partial derivatives of the objective function M to the variables a_n. Using

$$\begin{aligned}\frac{\partial M}{\partial\varphi(x,y)}&=\sum_{\xi,\eta}w(\xi,\eta)\left[F^*(\xi,\eta)\frac{\partial F(\xi,\eta)}{\partial\varphi(x,y)}+F(\xi,\eta)\frac{\partial F^*(\xi,\eta)}{\partial\varphi(x,y)}\right]\\&=-i\psi_c(x,y)\sum_{\xi,\eta}w(\xi,\eta)F^*(\xi,\eta)\exp\left[-\frac{2\pi i}{N}(x\xi+y\eta)\right]\\&\quad+i\psi_c(x,y)\sum_{\xi,\eta}w(\xi,\eta)F(\xi,\eta)\exp\left[\frac{2\pi i}{N}(x\xi+y\eta)\right]\\&=2\mathrm{Im}\{\psi_c(x,y)DFT[w(\xi,\eta)F^*(\xi,\eta)]\}\end{aligned} \tag{10}$$

We could calculate the partial derivatives of M with respect to a_n, expressed as

$$\begin{aligned}\frac{\partial M}{\partial a_n}&=\sum_{x,y}\frac{\partial M}{\partial\varphi(x,y)}\frac{\partial\varphi(x,y)}{\partial a_n}\\&=2\sum_{x,y}\mathrm{Im}\{\psi_c(x,y)DFT[w(\xi,\eta)F^*(\xi,\eta)]\}\cdot Z_n(x,y).\end{aligned} \tag{11}$$

The *fminunc* function in MATLAB could be used to perform the trust-region Newton algorithm and solve the unconstrained nonlinear optimization problem. Once the spectral energy metric M is maximized and the solutions of a_n are retrieved, we could generate the digital phase mask based on Eq. (4) and carry out the aberration compensation by Eq. (5).

3. Experimental verification

The availability of the proposed method is verified with experimental results. The living samples of mouse osteoblastic MC3T3-E1 cells are investigated with an off-axis digital holography system. The living cells are cultivated in a 35 mm Petri dish and suspending in liquid growth medium. The schematic setup of the imaging system is illustrated in Fig. 1. A frequency-doubled