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Joint Reconstruction Strategy for Telecentric-based Digital Holographic Microscopes

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Abstract: We present a reconstruction algorithm for digital holographic microscopy (DHM) operating in the telecentric regimen. This strategy rests on the minimization of a cost function to estimate both the numerical reference wave and the quantitative phase information. This algorithm paves the way to a universal DHM reconstruction tool.

1. Introduction

Digital holographic microscopy (DHM) has become in the last few years one of the most powerful quantitative phase imaging (QPI) techniques for study biological and non-biological samples. The hallmark of DHM systems is the capability to retrieve the complex wavefield (e.g., both amplitude and phase distributions) scattered by a sample using a label-free, non-invasive, single-shot method. In off-axis DHM, the accuracy of the reconstructed object information can be affected due to the presence of undesirable distortions associated with the imprecise estimation of the numerical reference wave [1], and/or a quadratic phase factor introduced by the microscope objective (MO) lens. The latter spherical phase factor can be optically eliminated when DHM systems do follow the telecentric configuration [2-4] (e.g., telecentric-based DHM systems). Previous reconstruction algorithms have been proposed to provide undistorted quantitative phase images based on the estimation of the optimal numerical reference wave [1, 5-7]. Common drawbacks of those approaches are their high computational effort, and/or their inaccessibility for direct users' use. In this work, we present a fast, accurate and automatic reconstruction algorithm for off-axis telecentric-based DHM systems. The proposed method is based on minimizing a cost-function to estimate jointly the numerical reference wave and an accurate quantitative phase image.

2. Proposed automatic method

Digital holographic microscopy is a hybrid imaging technique based on the optical recording of a hologram and its numerical reconstruction. DHM systems are optical interferometers such as Michelson and Mach-Zehnder interferometers. In the optical recording, the light beam emitted by a laser impinges on a beamsplitter. One of the split beams illuminates the sample, e.g., object wave, and the other split beam, which is a tilted plane wave with homogeneous amplitude, is the reference wave. In DHM, a sensor records the interference pattern between the reference wave, $r(\boldsymbol{x}) = \exp[\mathrm{i}(2\pi/\lambda)\sin\boldsymbol{\theta}\bullet\boldsymbol{x}]$ being $\boldsymbol{\theta} = (\theta_x, \theta_y)$ the tilted angle from the optical axis, and the complex wavefield diffracted by the sample, $o'(\boldsymbol{x})$. This interference pattern, commonly called hologram, is given by $h(\boldsymbol{x}) = |o'(\boldsymbol{x})|^2 + |r(\boldsymbol{x})|^2 + o'(\boldsymbol{x})\bullet r^*(\boldsymbol{x}) + o'^*(\boldsymbol{x})\bullet r(\boldsymbol{x})$, where $\boldsymbol{x} = (x, y)$ are the lateral spatial coordinates, $|\bullet|^2$ is the square modulus, and * is the complex conjugate operator. Both the amplitude and phase object information are reconstructed after retrieving the term $o'(\boldsymbol{x})$ from the recorded hologram. The reconstruction stage is easily understood by analyzing the hologram's spectrum. The Fourier Transform of the hologram can be estimated as $H(\boldsymbol{u}) = \mathrm{DC}(\boldsymbol{u}) + O(\boldsymbol{u} - \sin\boldsymbol{\theta}/\lambda) + O^*(\boldsymbol{u} + \sin\boldsymbol{\theta}/\lambda)$ where $\boldsymbol{u} = (u, v)$ are the lateral spatial frequencies, λ is the laser's wavelength, $\mathrm{DC}(\boldsymbol{u})$ is the zero-order of diffraction, and the second and third terms encode the spectrum of complex object information. If the DHM system operates in off-axis configuration (e,g., there is no overlay between the three terms composing the hologram spectrum), one can apply a spatial filter to extract $O(\boldsymbol{u} - \sin\boldsymbol{\theta}/\lambda)$ or $O^*(\boldsymbol{u} + \sin\boldsymbol{\theta}/\lambda)$ [8], which corresponds to the +1 and -1 term, respectively. Whereas the +1 term is located around the spatial frequencies $\boldsymbol{u} = \sin\boldsymbol{\theta}/\lambda$, the -1 term is centered around the spatial frequencies $\boldsymbol{u} = -\sin\boldsymbol{\theta}/\lambda$. To reconstruct the phase distribution of the object without any distortion, these terms must be properly centered to the frequency origin. Note that this lateral displacement is introduced by the tilted reference beam. Thus, for QPI-DHM measurements, one must compensate the reference tilt. In the real space, this compensation can be performed by multiplying the inverse Fourier transform of $O(\boldsymbol{u} - \sin\boldsymbol{\theta}/\lambda)$, and a digital replica of the reference wave, $r_D(\boldsymbol{x})$, commonly called as the numerical reference wave. Considering that the hologram is recorded by a discrete sensor (e.g., digital camera) with $M \times N$ square pixels of Δ_{xy} μm side, the discrete numerical reference wave $r_D(m, n)$ is expressed as

$$r_D(m,n)=\sum_{m,n}\exp\left[\mathrm{i}\frac{2\pi}{\lambda}\left(m\bullet\sin\theta_x\bullet M+n\bullet\sin\theta_y\bullet N\right)\Delta_{xy}\right], \tag{1}$$

where (m, n) are the discrete lateral coordinates of the sensor. Equation (1) shows that the computation of the numerical digital reference wave requires the precise knowledge of the interference angle, $\boldsymbol{\theta}=(\theta_x,\theta_y)$ and the sensor's features (e.g., number of pixels and pixel size). For a tilted reference plane wave, the interference angle can be estimated as $\theta_x=\sin^{-1}\left(\left|u_0-u_{max}\right|\lambda/M\Delta_{xy}\right)$, and $\theta_y=\sin^{-1}\left(\left|v_0-v_{max}\right|\lambda/N\Delta_{xy}\right)$, where (u_0,v_0) and, $(u_{\max},v_{\max})$ are the lateral pixel positions of the center of the DC, and ±1terms, respectively. Theoretically, the positions for $(u_{\max},v_{\max})$ should be easier to find since the Fourier transform of a plane wave is a Dirac delta. Thus, the Fourier transform of the hologram should have three maximum located at positions, (u_0,v_0) and $(\pm u_{\max},\pm v_{\max})$. However, due to the discretization introduced by digital camera, the position of $(\pm u_{\max},\pm v_{\max})$ may be a non-integer pixel. We propose to estimate the non-integer position of $(u_{\max},v_{\max})$ by minimizing a cost function that quantifies the best reconstructed phase image. The best reconstructed phase image is the one in which no remaining phase distortions are present. In other words, the best reconstructed phase image should be free of phase perturbations (e.g., no dark pixels in the binarized reconstructed phase image), and, therefore, the sum of all pixel values that compose the binarized reconstructed phase images will be given by the size of the phase image, $M\times N$ [1]. Based on this observation, we have defined the following cost function as $J=M\bullet N-\sum_{p=1}^{M}\sum_{q=1}^{N}\text{binarization}(\phi_{\hat{o}'})$, where $\phi_{\hat{o}}$ is the reconstructed phase image. **Figure 1** shows the best reconstructed phase image and a non-optimal reconstructed phase image of human red blood cells. For each reconstructed phase image, we have estimated the cost function J, value reported in Fig. 1. As expected, the best reconstructed phase image provides the lower value of the cost function, J = 1242 versus 5636.

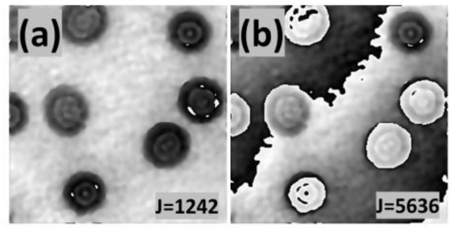

Fig. 1. Validation of the cost function to reconstruct phase images: (a) optimal compensation, (b) non-optimal compensation.

3. Conclusions

We have presented a fast and automatic method to reconstruct accurately the quantitative phase distribution of holograms recorded in an off-axis DHM system operating in a telecentric configuration. The input parameters of the proposed approach are only the laser's wavelength and the sensor's pixel size. The error difference between the experimental and nominal phase values is 0.54%, verifying the high accuracy of the proposed method to quantify phase measurements. Another advantage is the computational speed. The proposed method performs 40× faster than the previously-reported automatic approach based on *for*-loops [1]. The proposed method has the required features (e.g., fast, automatic, and accurate method) to become the universal reconstruction tool in QPI-DHM.

4. References

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