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Evaluation of the robustness and accuracy of PCA-based algorithms for in-line Digital Holographic Microscopy

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Abstract: This research undergraduate study evaluates the accuracy and robustness of a PCA-based phase reconstruction algorithm based on the number of phase-shifted images and the phase step for an in-line digital holographic microscope.

1. Introduction

A digital holographic microscope (DHM) is an optical interferometer in which one inserts a microscopic imaging system in one of the two arms to record the interference pattern between a uniform reference wave and the light scattered from a microscopic object. The advantages of DHM systems over different microscopic imaging modalities is the recording of the complex object distribution, enabling the reconstruction of amplitude and phase images of unprocessed (e.g., unstained) samples using a computational reconstruction algorithm. An incorrect selection and/or implementation of the reconstruction algorithms may lead to distorted and innacurate amplitude and phase measurements. The selection of the correct reconstruction algorithm can be provided by identifying the angle between the reference and object waves, classifying the DHM system as in-line, slightly off-axis, or off-axis [1-2]. In in-line DHM systems, the three diffraction orders that compose the hologram spectrum are completely overlapped, hence phase-shifting techniques are need to recover the complex object information, imposing the need for multiple hologram recordings [2]. Conventional phase-shifting DHM (PS-DHM) methods require the accurate knowledge of the phase shifts between the recorded holograms. However, such accuracy is frequently arduous experimentally, leading to the use of inexact values of the phase shift which produce distorted and unreliable phase maps. As an alternative, blind phase-shifting algorithms have been proposed to reconstruct amplitude and phase images without prior knowledge of the phase shifts [3-5]. In 2011, Vargas et al. proposed an approach based on Principal Component Analysis (PCA) to reconstruct both amplitude and phase distributions [6]. In this research undergraduate project, we investigate the performance of the PCA method for an in-line DHM system. In particular, we have evaluated the accuracy and robustness of PCA-based phase reconstruction algorithms based on the number of phase-shifted images and the value of the phase step. The PCA algorithm has been tested under noiseless and noisy conditions.

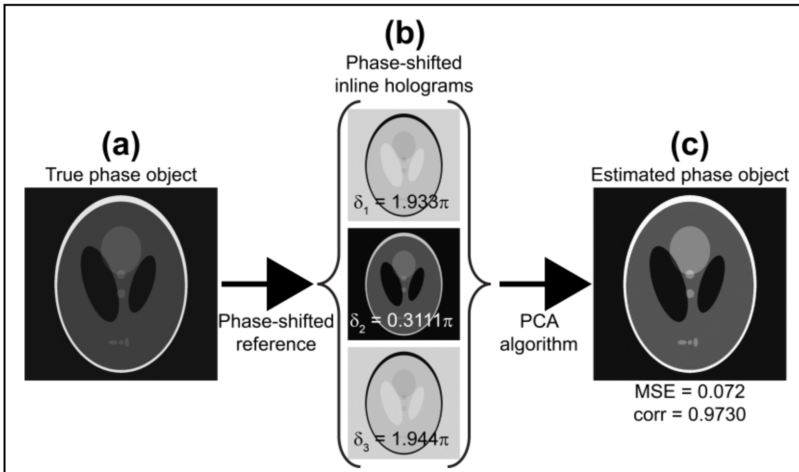

Fig. 1. Illustration of the PCA-based reconstruction phase algorithm for n phase-shifted inline holograms. The panels are: (a) true phase distribution; (b) 3 phase-shifted holograms; (c) estimated phase map after applying the PCA method [ref]. The phase step introduced by the reference wave was 1.933π, 0.3111π, and 1.944π. The mean square error (MSE) between the true and estimated phase distribution was 0.072. The correlation between both images is high (correlation value is 0.9730).

2. PCA-based framework

The PCA-based phase reconstruction method is based on the use of asynchronous phase-shifted holograms. In in-line DHM system, the irradiance distribution of a hologram (h) is composed of the sum of two terms, $h_n(x, y) = a(x, y) + b(x, y) \cos[\phi(x,y) + \delta_n]$, where (x, y) are the lateral spatial coordinates, $a(x, y)$ is the background illumination, $b(x, y)$ is the distribution of the fringes' contrast, $\phi(x,y)$ is the phase distribution, and δ_n is the phase shift(e.g., phase step) introduced by the reference wave. Expanding the cosine of the sum of two angles, the hologram distribution (h) can be rewritten as $h_n(x, y) = a(x, y) + b(x, y) \cos[\phi(x,y)] \cos[\delta_n] - b(x, y) \sin[\phi(x,y)] \sin[\delta_n]$. Therefore, the normalized hologram distribution is decomposed by two uncorrelated quadrature signals: h'_n

$(x, y) = h_n(x, y) - a(x, y) = b_{c,n}(x, y)\cos[\delta_n] + b_{s,n}(x, y)\ \sin[\phi(x,y)]$ being $b_{c,n}(x, y) = b(x, y)\cos[\phi(x,y)]$ and $b_{s,n}(x, y) = -b(x, y)\sin[\phi(x,y)]$. PCA methods reduce the dimensions of possibly correlated signals by selecting uncorrelated signals (e.g., the principal components). After applying the PCA-based algorithm proposed for phase-shifting interferometry [6], the sample phase distribution can be estimated as $\phi(x,y) = \tan^{-1}[b_{s,n}(x, y) / b_{c,n}(x, y)]$.

We have evaluated the PCA-based algorithm using simulated phase-shifted hologram. Figure 1(a) is the phantom phase image used to generate the phase-shifted holograms [panel (b)]. These phase-shifted holograms are the input images for the PCA-based algorithm, reconstructing the phantom phase distribution in Fig. 1(c). The quality of the estimated phase map is quantified by the mean-square error (MSE) and correlation values between the true and estimated phase maps. Both values are reported in Fig. 1. We have also reported the phase step between the phase-shifted in panel (c). Note that quality of the estimated phase distribution is highly dependent on the phase steps. Incorrect values of the phase steps lead to imprecise phase measurements. Figure 2 shows that three different phase-shifted holograms provide an estimated phase map with reduced accuracy (e.g., lower MSE and correlation values).

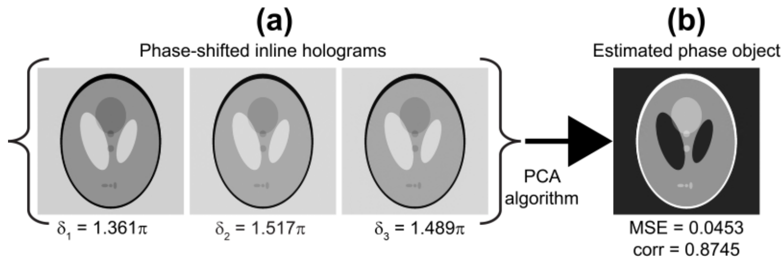

Fig. 2. Demonstration of the dependence between the phase steps and the quality of the estimated phase distribution. The use of incorrect phase steps leads to an imprecise phase measurement.

In this study, we have evaluated the quality of the PCA-based reconstructed phase images using 2, 3, 5, 7, 9, 11, 15, and 20 phase-shifted in-line holograms. Since the performance of the PCA algorithm depends on the selected phase steps, for each number of phase steps, we have reconstructed 100,000 phase maps for different combinations of the phase steps. In each combination, the value of the phase steps is randomly generated from 0 to 2π. Our study demonstrates that: (1) the minimum number of phase-shifted holograms for the PCA-based algorithm is 3; and (2) accurate phase images with an MSE value lower than 0.001 are only reconstructed for specific phase-steps combinations. For example, only 2,260 phase-steps combinations from the 100,000 possibilities provide an accurate estimated phase map, leading to a success rate of 2.26%. The highest success rate is obtained with 9 phase-shifted holograms, being equal to 2.97%. We have not been able to identify a relationship between the phase steps in the successful cases.

Finally, we have investigated the sensitivity of the PCA-based algorithm for noisy conditions. Noisy phase distributions are generated by adding a white Gaussian distribution to the true phase map [Fig. 1(a)] with different signal-to-noise ratio (SNR). The SNR has been changed from 5 dB to 45 dB. To evaluate the performance under noise, we have selected one successful case for 3 and 15 phase-shifted images. Whereas the PCA algorithm is not suitable for noisy holograms with SNR lower than 10 dB, the method is quite robust under noisy conditions with SNR higher than 15 dB, independently of the number of phase-shifted images.

3. Conclusions

This research undergraduate study shows that the PCA method developed by Vargas et al. in 2011 [6] is not a deterministic quantitative phase reconstruction tool for in-line DHM systems. The quality of the estimated is highly dependent on the phase steps. The reduced success rate of the PCA method restricts its experimental applicability as blind phase-shifting strategy in DHM.

4. References

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