Two-Wavelength Quantitative Phase Unwrapping of Dynamic Biological Processes

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Abstract: We present two-wavelength phase unwrapping system designs on asynchronous and dynamic phase microscopy techniques developed in our laboratory. The use of two wavelengths in these systems enables quantitative phase imaging without 2π-ambiguities.

OCIS codes: (180.3170) Interference Microscopy, (110.5086) Phase Unwrapping.

1. Introduction

Interferometric phase measurements at imaged points allow the detection of nanometer-scale optical path length changes of live cells on a biologically relevant time scale [1]. Methods of recovering complex phase information from interferometric data include acquisition of multiple phase-shifted images separated in time [2-3] or space [4], digital holographic wave front reconstruction [5], and also Hilbert transform processing [6]. All of these methods, however, yield phase images that include 2π ambiguities at points where the sample is optically thicker than the illumination wavelength. While various iterative unwrapping algorithms can be used to remove these ambiguities [7], multiple-wavelength optical phase unwrapping applied to digital holography effectively extends the range over which the phase image is continuous [8].

We implement a two-wavelength phase unwrapping technique on two different phase microscopy systems developed in our laboratory: asynchronous and dynamic phase microscopes. Both systems are based on digital holography interferometric setups and in both systems one acquires two phase shifted interferograms to eliminate common phase noise. However, in the first system the two phase-shifted interferograms are separated in time, whereas in the second system they are separated in space. Asynchronous digital holography [3] uses two near-simultaneous phase-shifted interferograms to recover phase information. Using the off-axis interferometer shown in Fig.1a, we obtain phase images of fibroblast cells free of 2π ambiguities as shown in Fig. 1b. This method still requires multiple acquisitions in time and contains artifacts due to interference fringe blurring.

Fig. 1. (a) Two-wavelength asynchronous digital holography microscope; (b) Quantitative phase map of a fibroblast cell produced using two-wavelength unwrapping.

2. Two-wavelength phase unwrapping applied to dynamic phase microscopy

Dynamic phase microscopy [4] system is based on an off-axis Mach-Zehnder interferometer as seen in Fig. 2a. Each laser is linearly polarized at 45° and coupled into a port of a beam splitter (BS1). The reference arm is converted to circularly polarized light by a λ/4 wave plate while the sample polarization is maintained at 45°. Light passing through the sample is magnified and imaged to the aperture plane (A) using a microscope objective (MO) positioned in a 4f configuration with lens L2. The reference arm light is expanded to fill the field of view using a 4f configuration of L1 and L2.

The 4f image-splitting system in Fig. 2b transforms the image at the aperture to the plane of the camera. The aperture reduces the field of view to less than ¼ of the camera sensor size. While between lenses L3 and L4, a diffraction grating separates the two laser wavelengths vertically and a Wollaston prism (W.P.) creates a horizontal angular separation between the two orthogonal polarizations. Thus, the 4f image-splitting system simultaneously projects four interferograms onto the camera: one at each wavelength for each phase-shifted polarization.

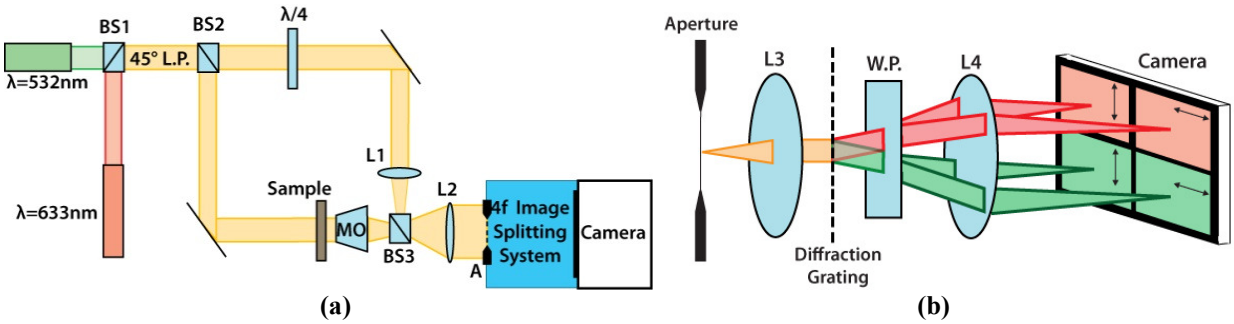

Fig. 2. (a) Two-wavelength dynamic phase-shifting microscope; (b) 4f image splitting system based on both polarization and wavelength splitting..

The off-axis interferometer produces a vertical fringe pattern with spatial frequency φ_c. Two interferograms, I_1 and I_2, acquired for each wavelength of illumination, have a relative phase shift α in their fringe patterns. Subtraction of the two interferograms yields a new interferogram that is free of DC and common noise. Using a Hilbert transform (HT) to recover the complex information in the signal allows computation of the phase at each point in the image, as seen below:

$$G = [\, I_1 - I_2 + j * HT\{I_1 - I_2\}\,] * \frac{e^{-j\varphi_c}}{1-e^{j\alpha}}; \quad \varphi_{OBJ} = \tan^{-1}\frac{\text{Im G}}{\text{Re G}}$$

The resulting phase image still contains discontinuities arising from 2π ambiguities in phase, which can be removed using two-wavelength unwrapping. Combining phase images of the same object that have been acquired with different illumination wavelengths λ_1 and λ_2 yields a new phase image that contains no phase ambiguities over the range of $\Lambda = \lambda_1\lambda_2/|\lambda_1 - \lambda_2|$ [7]. While this process amplifies the background noise of the image, the resulting unambiguous phase image can then be used as a guide to correct the phase of each original low-noise phase image. Further noise reduction can be obtained by averaging the corrected phase images from each illumination source.

3. Results and Conclusions

Figure 3 presents unwrapped phase images that contain artifacts due to the unwrapping process. Water droplets on a coverglass are shown in Fig. 3a. Figure 3b displays an MDA-MB-468 human breast cancer cell imaged using 633nm illumination on the dynamic phase microscopy system presented in [4]. These images demonstrate the ability of this system to produce quantitative phase images with very low background noise in a single acquisition. However, as indicated by the arrows, artifacts from the unwrapping algorithm implemented remain in the final phase image. Our future experiments will demonstrate the implementation of two-wavelength unwrapping with the dynamic phase microscopy system as a method of recovering the unambiguous phase profile without introducing such artifacts.

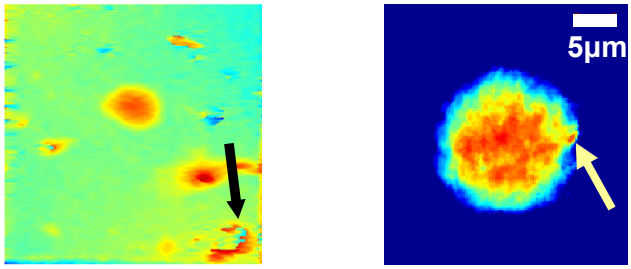

Fig. 3. Phase images containing 2π ambiguities after algorithmic phase unwrapping: (a) Water droplets; (b) MDA-MB-468 human breast cancer cell obtained by the system presented in Ref. [4].

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