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Laterally differential interference microscopy to observe phase objects

Jinhee Cho[1], and Ki-Nam Joo[1,#]

1 Dept. of Photonic Engineering, Chosun University, 309 Pilmun-daero, Dong-gu, Gwangju, 61452, South Korea
Corresponding Author / Email: knjoo@chosun.ac.kr, TEL: +82-62-230-7235, FAX: +82-62-230-7440

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In this investigation, we propose a laterally differential interference microscopy to be compact and enable a snapshot measurement. The proposed microscopy adopts the lateral shearing interferometric principle, which can obtain the lateral slope of the measurement wave from the self-interference without the reference. Due to the absence of the reference, the system is more stable than the typical interferometric systems. It uses a polarization grating to generate two laterally shifted wavefronts, which makes the system more compact and reliable, because of its birefringent, polarizing beam splitting characteristics. Furthermore, the use of a polarization camera does not require sequential measurements for the phase extraction. In the experiments, we observed and measured the temporal changes of various specimens to verify the system performance. Because the proposed microscopy also has the benefit to be adaptable to the typical microscopy system instead of using an imaging camera, it can be replaced with the conventional contrast microscopy.

1. Introduction

In biomedical fields, microscopy is an essential tool to observe cells and features in the tissues. Typically, a wide-field microscopy can collect the light from the samples, and image them in the focal region to obtain their clear characteristics. However, lots of samples are phase objects, which change the phase but not the amplitude of light contrary to amplitude objects. Because of no intensity difference, the wide-field microscopy has limitation to clearly observe the target and cannot characterize it. In order to overcome this limitation, differential interference contrast microscopy (DIC) or phase contrast microscopy (PCM) has been developed, and its capability to obtain the intensity variations caused by the phase enables to successfully distinguish phase objects. One of the drawbacks is that its main functionality is for the observation of the samples, however, not quantitative analysis, because these intensity variations are mixed with other background intensity, making it difficult to extract quantitative information.

On the other hand, a quantitative phase imaging (QPI) relevant to interferometric analysis has been proposed [1,2] and implemented with various structures such as Michelson-, Mach-Zehnder-, and Sagnac types of optical configuration to obtain the phase image independent of the background intensity. In spite of their obvious measurement results to quantitatively characterize the phase objects, however, the system becomes quite complicated and time-consuming to obtain phase information from the images. These limitations of the system restrict the applicability and stability.

In this investigation, we propose a laterally differential interference microscopy (LDIM) to be compact and enable a snapshot measurement. The LDIM adopts the lateral shearing interferometric principle, which can obtain the lateral slope of the measurement wave from the self-interference without the reference. Due to the absence of the reference, the LDIM is more stable than the typical interferometric systems. The LDIM uses a polarization grating to generate two laterally shifted wavefronts [3], which makes the system more compact and reliable, because of its birefringent, polarizing beam splitting characteristics. Furthermore, the use of a polarization camera (PCMOS) does not require sequential measurements for the phase extraction. The PCMOS, where a polarizer array with 0°, 45°, 90° and 135° transmission axes is put on the imaging sensor, can obtain four phase-shifted interferograms at once to calculate the phase image based on the spatial phase shifting technique.

2. Laterally differential interference microscopy

2.1 Optical configuration of laterally differential interference microscopy using a polarization grating

Figure 1 shows the optical configuration of the laterally differential interference microscopy (LDIM), which consists of a polarization grating (PG), a mirror (M) and a polarization pixelated CMOS camera (PCMOS). As an optical source, a broadband is used,

and the reflected beam from the specimen is incident to the PG. The incident beam is split into two beams by the PG, and the returning beams can be laterally shifted after reflecting off the flat mirror and passing through the PG again. These two beams are not only laterally shifted, but also their polarization states are orthogonal to each other as circular polarizations (right-handed circular polarization, RHCP and left-handed circular polarization, LHCP).

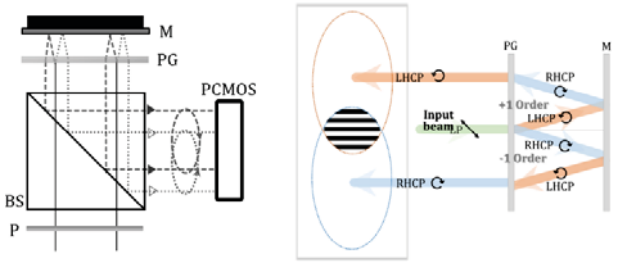


Fig. 1 Schematic of the laterally differential interference microscopy; P, linear polarizer; BS, beam splitter; PG, polarization grating; M, mirror; PCMOS, polarization pixelated CMOS camera; RHCP, right-handed circular polarization LHCP, left-handed circular polarization.

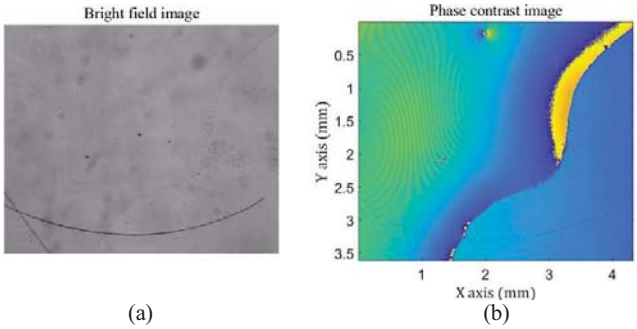
2.2 Instantaneous phase extraction

When two circularly polarized (RHCP and LHCP) beams are incident to the PCMOS, four kinds of interferograms can be obtained by the array of linear polarizers as

$$\begin{aligned} I_0 &= A[1+\gamma sin(\Delta W_L)] \\ I_{45} &= A[1+\gamma cos(\Delta W_L)] \\ I_{90} &= A[1-\gamma sin(\Delta W_L)] \\ I_{135} &= A[1+\gamma cos(\Delta W_L)] \end{aligned} \quad (1)$$

where I_0, I_{45}, I_{90} and I_{135} are the interferograms detected at the PCMOS pixels with 0°, 45°, 90° and 135° rotated linear polarizers, respectively. A is the background intensity and γ indicates the fringe contrast. Because of the lateral shearing interference, the lateral phase slope (ΔW_L) is included in the interferograms, which are $\pi/2$ phase-shifted as known in Eq. (1). Then, ΔW_L can be simply calculated by the four-step phase shifting algorithm as

$$\Delta W_r = tan^{-1}\left(\frac{I_0 - I_{90}}{I_{45} - I_{135}}\right) \quad (2)$$



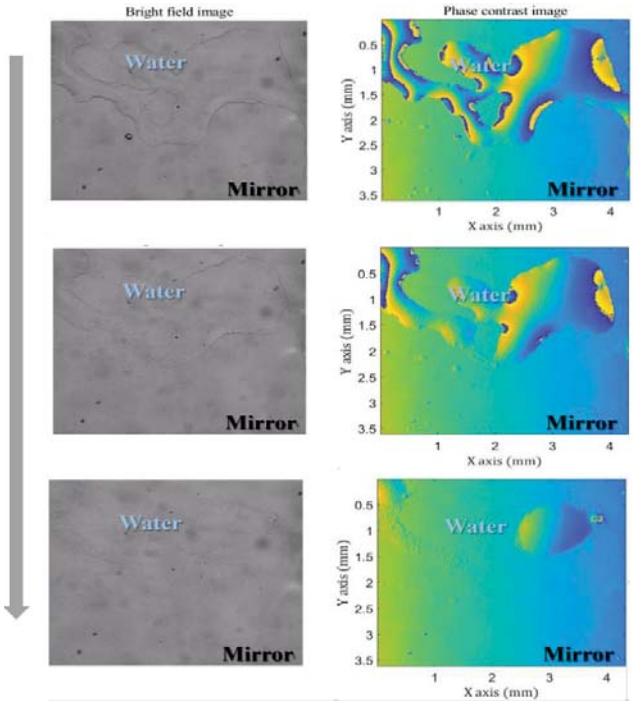
(a) (b)

Fig. 2 (a) Bright field image and (b) phase contrast image of the target consists of a plane mirror and water on it.

3. Experimental results

Figure 2 shows the feasibility test result of the LDIM. As a target, a plane mirror was prepared and a small amount of water was drop on the plane mirror. As shown in Fig. 2(a), the water was not clearly detected in the bright field image, but the phase image of the target obtained by the LDIM shows the water layer on the mirror. In addition, the thickness of the water film was also observed by the phase change.

Further experiments to verify the dynamic imaging capability was also implemented. The similar target (water on the plane mirror) was prepared, and the phase contrast images were capture in real time. As shown in Fig. 3, the evaporation of the water on the plane mirror was clearly observed while it was not in bright field microscopy.



(a) (b)

Fig. 3 Captured (a) bright field images and (b) phase contrast images of the target consists of a plane mirror and water on it during evaporation of water film.

4. Conclusion

We proposed a laterally differential interference microscopy (LDIM), which adopts the lateral shearing interferometric principle. The LDIM uses a polarization grating to generate two laterally shifted wavefronts, which makes the system more compact and reliable. In the experiments, we observed and measured the temporal changes of various specimens to verify the system performance. Because the LDIM has the benefit to be adaptable to the typical microscopy system instead of using an imaging camera, it can be replaced with the conventional contrast microscopy.

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