Dynamic Quantitative Phase Microscopy of Biological Cells

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Abstract: We introduce a new fast and accurate method for dynamic quantitative phase imaging of biological cells. The method enables imaging of sub-millisecond dynamic biological phenomena with sub-nanometer temporal stability. Initial experimental results are given.

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Imaging living cells requires a system that is able to acquire mostly transparent three-dimensional objects, with very little inherent absorption, imposing almost no change on the light amplitude reflected from or transmitted through them. Imaging the phase of light transmitted through these transparent objects gives an indication of the optical path delays associated with them. Phase imaging can be performed by several well-known microscopy techniques, such as the phase contrast microscopy and differential interference contrast microscopy [1]. However, these conventional microscopy techniques do not yield quantitative phase measurements. In addition, they suffer from various artifacts that make it hard to correctly interpret the resulting phase images in terms of optical path delays. Digital holography, on the other hand, is able to yield quantitative measurements of the phase distribution [2,3]. Therefore, it is possible to reshape the captured complex wave front and, for example, compensate for different optical aberrations. Digital holography, however, requires interferometric setups, typically yielding phase images that are not clear from phase noise. Eliminating most of the phase noise can be obtained by acquiring two or more interferograms of the same sample [4,5]. Still, certain biological processes, such as cell membrane fluctuations and neuron activity, occur faster than the scanning rates of most optical systems. Therefore, this paper suggests a new optical system for obtaining dynamic quantitative phase measurements with high precision and low degree of phase noise. Using a new double-channel interferometric setup, two phase-shifted interferograms of nearly-transparent biological samples are acquired, in a single digital camera exposure, and digitally processed to yield the phase profile of the sample. Since two interferograms of the same sample are acquired simultaneously, most of the common phase noise is eliminated, enabling the visualization of sub-millisecond dynamic cell phenomena with sub-nanometer temporal stability of the phase profile.

The suggested system, shown in Fig. 1(a), includes an off-axis digital holographic microscopy setup based on a Mach Zehnder interferometer, followed by an image splitting system. Light from a HeNe laser source, linearly-polarized at 45°, is split into sample and reference beams by beam splitter BS_1. The sample arm includes a sample, and microscope objective MO that is in a 4f configuration with lens L_2. Lens L_1 in the reference arm is in a 4f configuration with lens L_2 as well. BS_2 combines the sample and reference beams, and the interference pattern of interest appears one focal length behind lens L_2. This interference pattern is then spatially restricted by an aperture to an area that is not bigger than half of the digital camera sensor size. The interference on the aperture plane is then imaged, through the 4f image splitting system shown in Fig. 1(b), onto the digital camera. This 4f image splitting system includes two identical lenses, L_3 and L_4, creating 1x magnification of the aperture plane onto the camera. A Wollaston prism is positioned between these two lenses to create two spatially-separated and phase-shifted interferograms on the camera.

The phase shift between the interferograms is explained in the following. In the reference arm, a quarter ($\lambda/4$) wave plate is oriented along the horizontal axis, so that the light transmitted through it has a 90° phase difference between the horizontal and vertical components. On the other hand, in the sample arm the light remains 45° linearly-polarized, so there is 0° phase difference between the horizontal and vertical components. When the beams are combined by BS_2, there is $\alpha = 90^\circ$ shift between the interference pattern formed by the horizontal component and the interference pattern formed by the vertical component. Due to its ability to output two perpendicularly-polarized beams, the Wollaston prism separates the horizontal or vertical components for each of the interferograms, yielding a phase shift of α between the interferograms. Once the two interferograms, I_1 and I_2, are acquired by the digital camera in a single exposure, the wrapped object phase ϕ_{OBJ} can be found as follows:

$$F = \left[I_1 - I_2 + j \cdot HT\{I_1 - I_2\}\right] \cdot \exp(-j\phi_C)/[1-\exp(j\alpha)]; \quad \phi_{OBJ} = \arctan\{\mathrm{Im}\,F/\mathrm{Re}\,F\}, \tag{1}$$

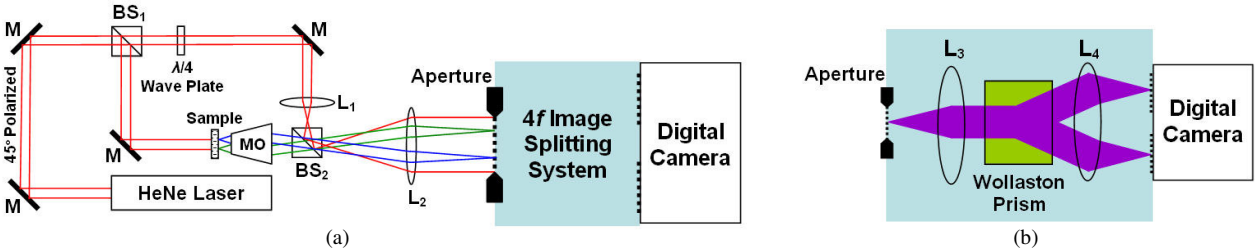

Fig. 1. Double-channel interferometer for obtaining phase profiles of biological cell dynamics: (a) The entire interferometer; (b) The 4f image splitting system.

where ϕ_C is the phase difference of the interferometer without the presence of the object, and *HT* denotes a Hilbert transform. ϕ_C and α can be digitally measured by fitting the background interference (interference pattern observed without the presence of the sample) in each of the interferograms to a sine wave. Then, an unwrapping algorithm can be applied to get the final unwrapped object phase. Note that the process described above removes most common noise and background elements, enabling reliable phase measurement. In addition, since the interferograms are acquired simultaneously, in a non-scanning manner, fast phenomena can be visualized.

We have implemented the optical system shown in Fig. 1. We used MDA-MB-468 human breast cancer cells as the sample to be imaged. Figure 2(a) shows the raw microscopic image of the sample, demonstrating the very low visibility of simple imaging of this sample. In both the reference and sample arms, we used 40x, 0.65 NA achromatic objective lenses. Each of these lenses created 33x magnification with lens L_2 (15 cm focal length). Then, the resulting complex amplitude was spatially restricted by a 3.5 mm × 2.3 mm aperture. The 4f image splitting system shown in Fig. 1(b) contained two similar lenses L_3 and L_4, each with a focal length of 7.5 cm. The Wollaston prism (crystal quartz, two degrees of angular separation), positioned between these two lenses, split the pattern from the aperture plane so that the CCD camera (AVO Pike F032-B, 640×480 pixels) captured two phase-shifted interferograms, I_1 and I_2, that were spatially separated by 2.4 mm. These two interferograms are shown in Fig. 2(b). A test target (USAF resolution chart) was used as a preprocessing sample to determine the coordinate mapping for splitting the single CCD image into two different interferograms. The frequency q and the phase shift α were extracted by fitting each of the interferograms to a sine wave. Based on the fact that the background interference fringes in each of the interferograms are vertical, we get $\phi_C = qx$, where x is the horizontal coordinate in each interferogram frame. Figure 2(c) shows the final unwrapped phase profile of the sample as obtained by applying Eq. (1) and a phase unwrapping algorithm. Recording the temporal path-length fluctuations associated with a diffraction-limited spot of the imaging optics at millisecond rates yields fluctuations with standard deviation of only 0.94 nm, indicating that sub-nanometer path-length sensitivity can be obtained within sub-millisecond time scale.

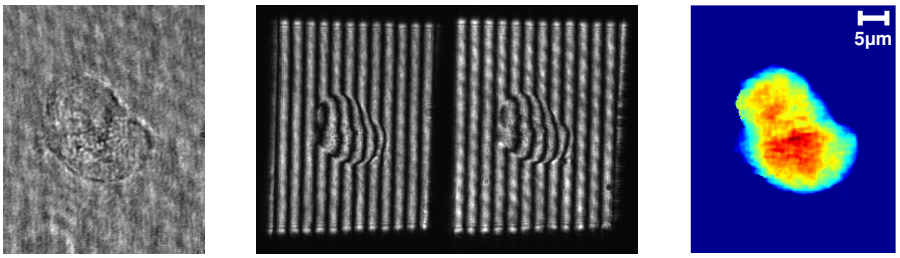

Fig. 2. Experimental results obtained from MDA-MB-468 human breast cancer cells: (a) Raw image; (b) The two phase-shifted interferograms captured in a single camera shot; (c) Final unwrapped phase profile.

To conclude, we have demonstrated a new method that is able to acquire fast biological phenomena in high accuracy. Initial experimental results show good spatial resolution for static cell phase imaging. Due to the simultaneous acquisition manner, capturing fast cellular phenomena is only limited by the true frame rate of the camera. This will be demonstrated in our near-future experiments.

References

[1] M. Pluta, Advanced Light Microscopy, Vol. 2 (Elsevier Science Publishing, 1988).
[2] T. Zhang and I. Yamaguchi, Opt. Lett. **23**, 1221 (1998).
[3] P. Marquet, B. Rappaz, P. J. Magistretti, E. Cuche, Y. Emery, and T. Colomb, and C. Depeursinge, Opt. Lett. **30,** 468 (2005).
[4] G. Popescu, L. P. Deflores, J. C. Vaughan, K. Badizadegan, H. Iwai, R. R. Dasari, and M. S. Feld, Opt. Lett. **29**, 2503 (2004).
[5] Kevin J. Chalut, William J. Brown, and Adam Wax, Opt. Express **15**, 3047 (2007).