Quantitative phase-shifting DIC using programmable spatial light modulators

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ABSTRACT

A quantitative phase-shifting Differential Interference Contrast (DIC) system is built using a programmable spatial light modulator (SLM). Our system offers halo-free phase gradient images with low illumination coherence and very good axial sectioning. Results are presented for standard polystyrene micro-beads and live cells.

Keywords: Quantitative DIC, halo-free, microscopy, programmable spatial light modulator

1. INTRODUCTION

Most biological specimens are transparent under bright-field inspection. These specimens are known to be "phase objects", which do not significantly absorb any power of the incident light. Instead, they retard phase fronts of the incident light. This phase retardation has been used to render visible contrast in phase-contrast microscopy as proposed by Zernike [1] based on interference phenomenon. Although high contrast is obtained, the use of partially coherent illumination and a phase ring with finite radial thickness, causes phase-contrast microscopy to suffer from the *halo* effect, in which bright areas appear surrounding dark objects and vice versa [1, 2]. On the other hand, DIC works with completely incoherent illumination and generates halo-free intensity images relating to the gradient of the phase front [3, 4]. However, to our knowledge, the quantitative aspects of DIC are not well characterized. Meanwhile, these aspects are well understood in the context of phase imaging [5-10]. Currently, most DIC systems are qualitative in the sense that they do not provide any quantitative information on the gradient of the wavefront. In this paper, we introduce a novel phase-shifting DIC setup using a single SLM. Although phase-shifting DIC is not new [11-13], this is the first time, to our knowledge, that SLMs are used for phase-shifting DIC. In other phase-shifting DIC work e.g. [12], the phase shift is obtained by mechanically translating the DIC prism. This displacement gives 4 values of the phase difference: $k\pi/2$, $k = 0,1,2,3$ between the two cross-polarized fields before they interfere. Then, four interference intensity images are used to calculate the phase gradient numerically. In our method, a SLM is used to control this phase difference. Because the SLM does not operate as a zero-order waveplate, we reflect the field at the SLM twice with the second reflection has a polarization directions rotated by 90^{o} as shown in Figure 1. The polarization axes of these fields are aligned with the modulating and non-modulating axes of the SLM. Our system serves as an add-on module to a commercial DIC microscope and requires minimal modification to existing DIC microscopes. It provides halo-free quantitative information on phase gradient image of the sample with high spatial and temporal stability and very good depth sectioning. Experimental results are shown for standard sample (e.g. polystyrene beads) and live Hela cells.

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2. METHOD

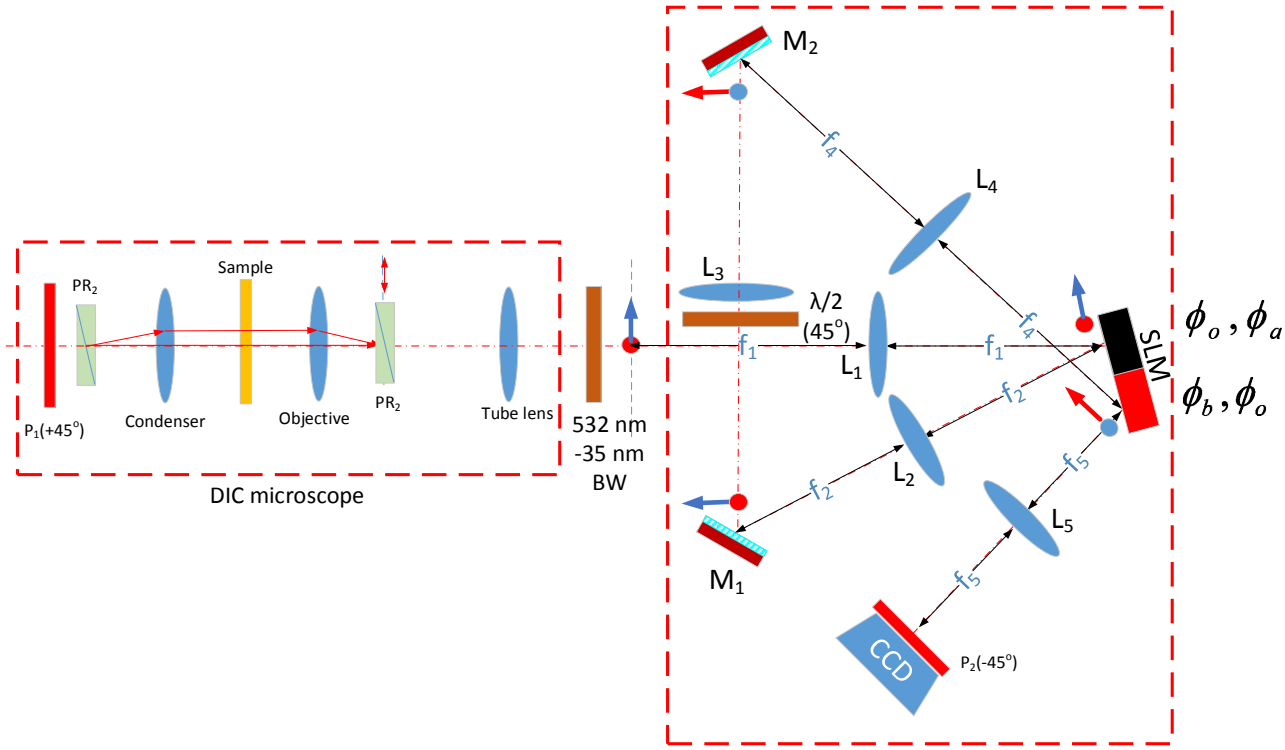

Figure 1. Proposed optical setup for the QDIC module

Our optical setup is given in Fig. 1. The system consists of a Nikon IX70 DIC microscope with a polarizer in the DIC configuration removed. This removal allows two slightly shifted, cross-polarized fields coming to t he o utput por t of the microscope, referred to as S-polarized and P-polarized fields. The phase difference between S and P is controlled by an external quantitative DIC (QDIC) module. This module consists of two 4f systems, one 1:1 mapping system and an SLM. The first 4f system, $L_1 - L$, maps the fields at the output port of the microscope onto the surface of mirror M_1. On the surface of M_1, S and P will be phase retarded by ϕ_a and ϕ_o by the SLM, respectively. Here, ϕ_a and ϕ_o are the phase shifts generated for the polarization along the modulating and non-modulating axes of the SLM. M_1, M_2 are placed at two times the focal length of lens L_3, forming a 1:1 mapping system where the surfaces of M_1 and M_2 are conjugated to each other. This system combines with a half waveplate oriented at 45^o to rotate the S, P fields by 90^o upon coming to the surface of mirror M_2. Consequently, the P-field now aligns with the modulating axis of the SLM instead of the S-field. The second 4f system, $L_4 - L_5$, further maps the S-P field onto the surface of the CCD. However, thanks to polarization rotation of the half waveplate, S and P fields will be further phase shifted by ϕ_o and ϕ_b. Here, ϕ_b is the phase shift generated by the modulating axis on the second half of the SLM (see the setup). As a result, the total phase shifts accumulated by S and P fields are $\phi_a + \phi_o$ and $\phi_o + \phi_b$, respectively. Hence, their relative phase difference is $\Delta\phi = \phi_b - \phi_a$. The polarizer P_2 allows these two fields to interfere on the CCD's surface. In our system, $\Delta\phi$ is set to 4 values $k\pi/2$, $k = 0,1,2,3$. Post-processing steps to extract phase gradient from intensity measurements are presented below.

Assuming the translation between the two replicas is along the $x-$ direction with a known amount of Δx. Measured intensity at a transverse location $\mathbf{r}$ on the detector plane with phase shift $\Delta\phi$, generated by the SLM, can be written as:

$$I(\mathbf{r};\Delta\phi) = \left|U(\mathbf{r}) + U(\mathbf{r}+\hat{\mathbf{x}}\Delta x)e^{i\Delta\phi}\right|^2 = 2 + 2\cos\left[\phi(\mathbf{r}+\hat{\mathbf{x}}\Delta x) - \phi(\mathbf{r}) + \Delta\phi\right]$$
$$\approx 2 + 2\cos\left[\Delta x \nabla_x \phi(\mathbf{r}) + \Delta\phi\right]. \quad (1)$$

Here, $\hat{\mathbf{x}}$ is the unit vector along the x-direction. We also assume phase objects so that $|U(\mathbf{r})| \approx |U(\mathbf{r}+\hat{\mathbf{x}}\Delta x)| \approx 1$. From Eq. (1), we can either specify the phase difference or the phase gradient along the $x-$ direction as

$$\Delta_x\phi(\mathbf{r}) = \Delta x \nabla_x \phi(\mathbf{r}) = \arctan\left[I(\mathbf{r};-\pi/2) - I(\mathbf{r};\pi/2); I(\mathbf{r};0) - I(\mathbf{r};\pi)\right],$$
$$\nabla_x\phi(\mathbf{r}) = \frac{1}{\Delta x}\arctan\left[I(\mathbf{r};-\pi/2) - I(\mathbf{r};\pi/2); I(\mathbf{r};0) - I(\mathbf{r};\pi)\right]. \quad (2)$$

3. RESULTS

Figure 2 shows phase difference images $\Delta_x\phi(\mathbf{r})$ of 3-micron polystyrene beads n = 1.5983 in dry air n = 1. To change the coherence of the illumination, we varied the numerical aperture of the condenser, NA_c, to three different values 0.125, 0.25 and 0.55. Corresponding phase difference images of the beads are shown in Fig. 2 a - c). Figure 2 d) shows the cross-sections of the phase difference along line profiles specified in Figure 2 a - c). It can be seen that smaller values of NA_c give larger values of the phase difference and vice versa. Note that this phase reduction was recently described in the context of QPI in [14].

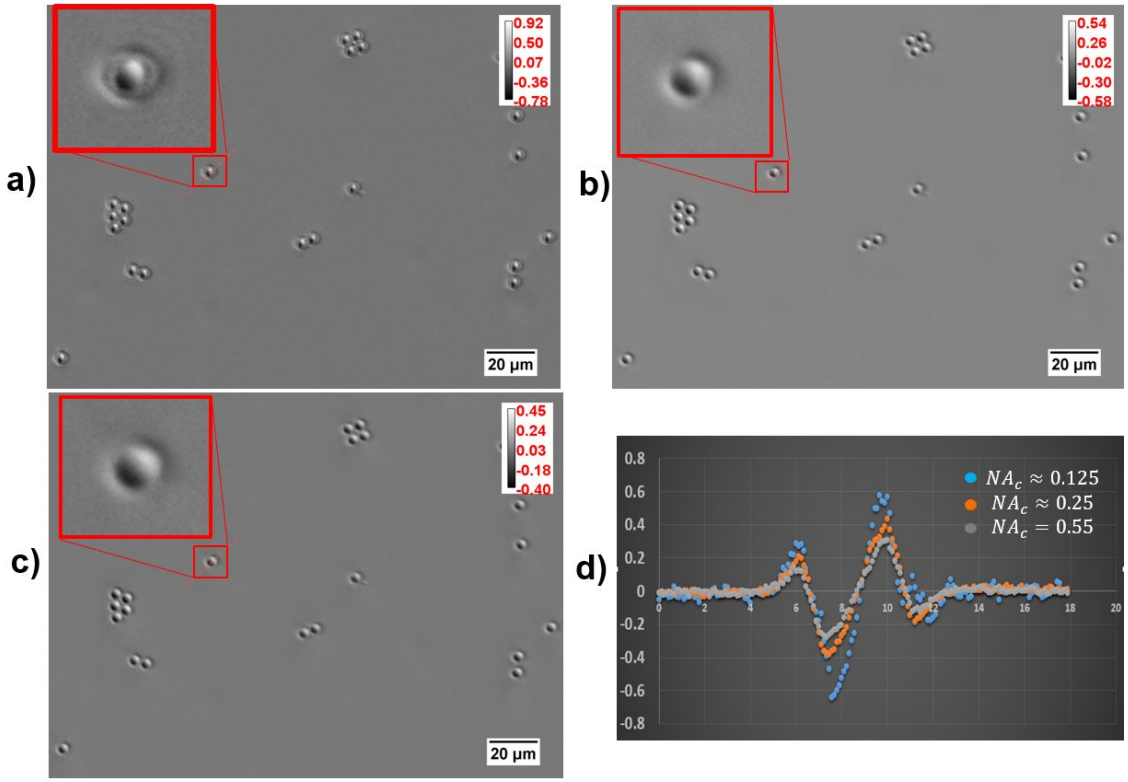

Figure 2. a - c) Quantitative phase difference images of 3-micron polystyrene beads in dry air at different values of NA_c. d) Cross-section values along the line profiles specified in a-c).

Next, we consider quantitative DIC imaging of live HeLa cells. The cells are thawed and cultured at about 30% confluence in a glass-bottom dishes (MatTek) with EMEM with 10% FBS. Then, they are passaged and left in the humidified and climate controlled incubator with 5% CO2 and 37°C. This step gives the cells the necessary time to attach and flatten at the bottom of a glass dish. Phase different images of a HeLa cell are shown in Figure 3 a- c) for various values of NA_c. It can be observed that when NA_c increases, the contrast of the phase different images reduces. However, all the details of the cell are still observable and the images are halo-free.

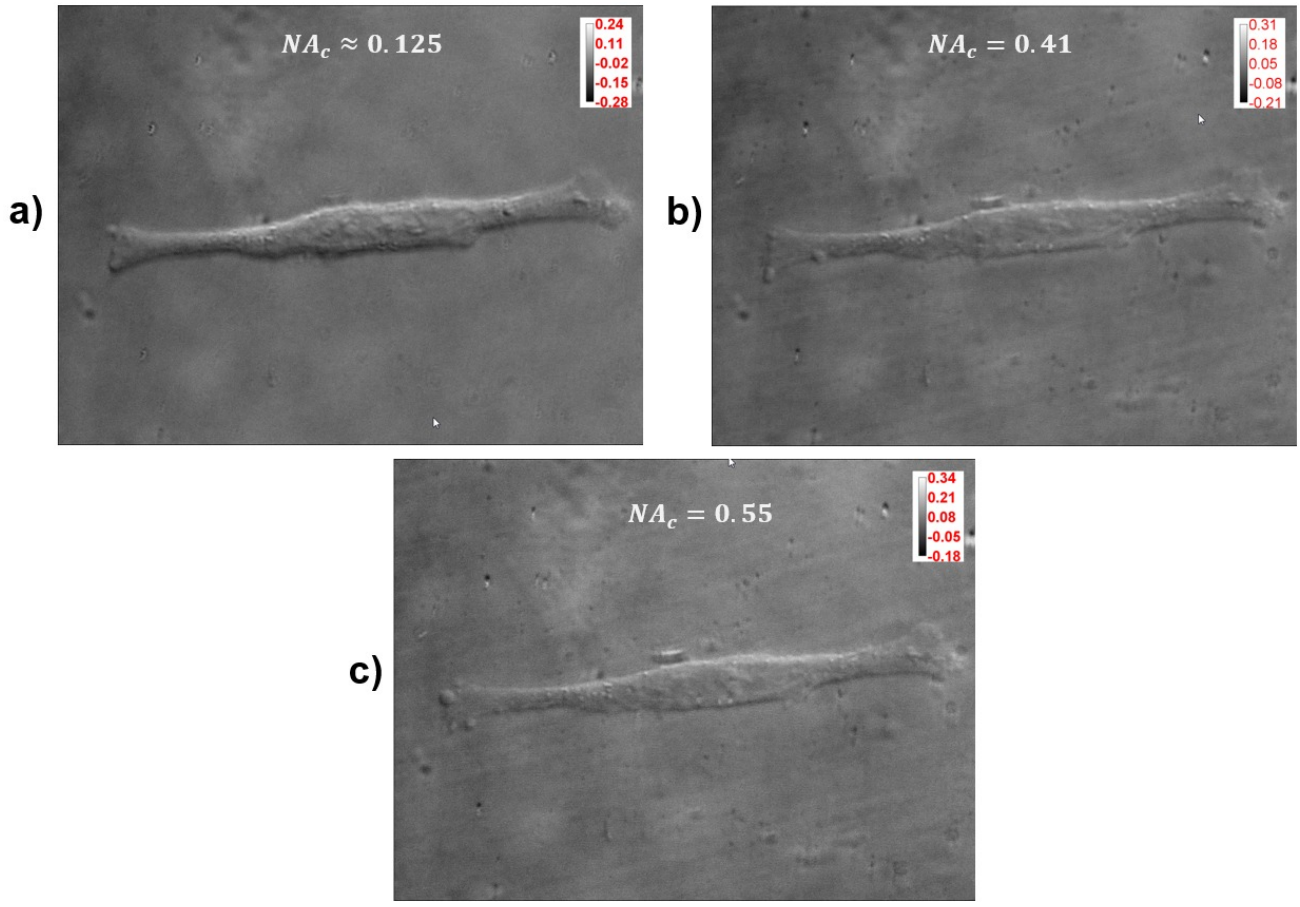

Figure 3. Phase different images of a Hela cells at different values of NA_c. The unit is radian.

4. CONCLUSIONS & FUTURE WORK

In this paper, we proposed a novel optical setup to obtain quantitative phase difference images and therefore, phase gradient image, of transparent sample. Our system requires minimal modification to existing DIC microscope. Here, an SLM is used to obtain various phase differences between two cross-polarized fields in a DIC system. Post-processing is applied to obtain the phase gradient using simple calculation. Our method is demonstrated on polystyrene micro-beads and live Hela cells. Experimental results show that although halo-free images are obtained, measured values of the phase difference reduce with the coherence of the illumination. Further work includes modeling this effect and performing post-correction to obtain correct quantitative values.

5. ACKNOWLEDGMENTS

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