

Quantitative DIC microscopy using an off-axis self-interference approach

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Traditional Normarski differential interference contrast (DIC) microscopy is a very powerful method for imaging nonstained biological samples. However, one of its major limitations is the nonquantitative nature of the imaging. To overcome this problem, we developed a quantitative DIC microscopy method based on off-axis sample self-interference. The digital holography algorithm is applied to obtain quantitative phase gradients in orthogonal directions, which leads to a quantitative phase image through a spiral integration of the phase gradients. This method is practically simple to implement on any standard microscope without stringent requirements on polarization optics. Optical sectioning can be obtained through enlarged illumination NA. © 2010 Optical Society of America
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Differential interference contrast (DIC) microscopy has long enjoyed widespread use in imaging transparent phase objects by the biological community. It directly converts changes of phase gradients in the sample to intensity changes. The advantages of DIC over other phase imaging techniques, such as phase contrast microscopy, include outstanding contrast, high spatial resolution, and optical sectioning capability. However, one of the major limitations of DIC microscopy is that it is qualitative in nature owing to the nonlinear relationship between intensity and phase gradient. Moreover, the amplitude information of the sample is convolved with the phase information, which sometimes makes data interpretation difficult.

Digital holography microscopy or quantitative phase microscopy has been developed over the past decade to overcome these problems [1–4]. Two different approaches have generally been employed. One is phase-shifting interferometry [4], which uses several precisely phase-shifted interferograms to recover the amplitude and phase of the sample. The other approach is off-axis interferometry, where the sample wavefront interferes with a tilted reference wave in off-axis geometry, enabling one-shot phase image retrieval [3]. Once the quantitative phase image is obtained, pseudo-DIC images can be simulated along any gradient direction by simply taking the derivative [5]. However, most of the reported works utilized highly coherent optical sources with pseudo-plane-wave illumination such that the phase images were prone to fixed pattern speckle noise, and sectioning capability was very poor.

On a different note, many researchers are pushing traditional Normarski DIC to obtain quantitative imaging through the use of a phase-shifting technique [6–8]. Similar to phase-shifting interferometry, this approach requires precise change of one sample arm (in this case, by adjusting the DIC bias) in three or four steps to convert

DIC intensities to linear phase gradients in one shear direction. Orthogonal directions of shear are necessary for reconstruction of object phase function. The whole process requires multiple adjustments of a phase compensation wave plate and rotation of the sample or Wollaston prisms and, therefore, is unsuitable for real-time imaging.

Here we present a unique approach for quantitative DIC microscopy that yields single-shot linear phase gradient images at two orthogonal directions using off-axis sample self-interference. By placing a Ronchi grating slightly out of focus at the image plane of a conventional microscope and then relaying the image onto the camera, the lateral shearing effect will give rise to interference fringes similar to off-axis interferometry [9]. Applying the digital holography algorithm enables single-shot phase gradient imaging. Quantitative DIC images along two orthogonal shear directions are obtained simultaneously using a checkerboard grating. This allows direct calculation of quantitative phase image through integration. We demonstrate quantitative DIC and phase imaging of living cells using this approach.

Our DIC microscopy setup consists of an Olympus Abbe condenser, a sample stage, a 100× oil-immersion objective, and a 15 cm achromatic lens as the tube lens (shown in Fig. 1). The sample was illuminated by a blue LED ($\lambda = 470$ nm) with a Köhler illumination configuration. A Ronchi grating or checkerboard grating is placed close to the image plane (IP1) of the microscope, which is imaged on a CCD camera (Photometrics Coolsnap) located at the second image plane (IP2) using a $4f$ imaging lens pair. As shown in the inset of Fig. 1, the grating splits the sample wavefront into multiple orders and only the first and zeroth orders are allowed to pass through a spatial filter mask. These two orders will interfere at IP2, where the CCD camera is located. Equation (1) describes the interferogram on the CCD under plane-wave illumination (when the condenser iris is almost closed):

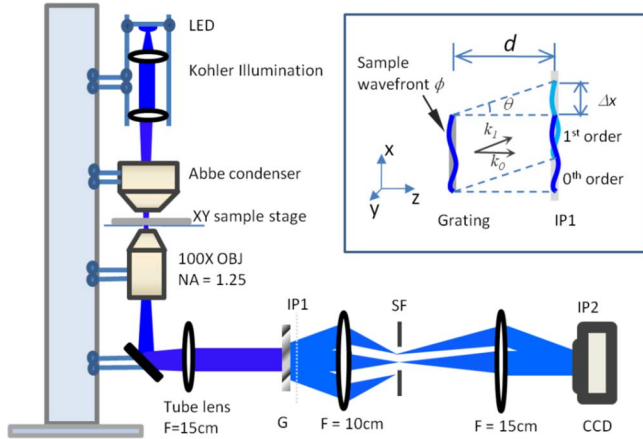


Fig. 1. (Color online) Schematic diagram of our off-axis DIC microscope setup. The inset shows the lateral shearing of the wavefront caused by the displacement of the grating from the image plane. IP1, IP2, image plane 1 and 2; G, grating; SF, spatial filter.

$$\begin{aligned}
 S &= |A_0(x, y)e^{-i(\vec{k}_0 \cdot \vec{x} + \phi(x, y))} \\
 &\quad + A_1(x + \Delta x, y)e^{-i(\vec{k}_1 \cdot \vec{x} + \phi(x + \Delta x, y))}|^2 \\
 &= A_0(x, y)^2 + A_1(x, y)^2 \\
 &\quad + 2A_0(x, y)A_1(x + \Delta x, y) \cos[(\vec{k}_1 - \vec{k}_0) \cdot \vec{x} \\
 &\quad + (\partial\phi(x, y)/\partial x)\Delta x], \quad (1)
 \end{aligned}$$

with $A_0(x, y)$, $A_1(x, y)$ being the amplitude of the zeroth- and first-order sample waves at sample position (x, y) , respectively; k_0 and k_1 are the corresponding wave vectors, and $\phi(x, y)$ is the optical phase of the wave after traversing the sample. The first term inside the cosine function forms the sinusoidal interference fringes, whereas the second term distorts the sinusoidal pattern and embeds the sample phase gradient information. Obviously the phase contrast depends linearly on the amount of lateral shift Δx , which can be calculated by using the following equation:

$$\Delta x_{\text{sample}} \approx \frac{d \sin(\theta)}{\text{Mag}} = \frac{d\lambda}{L\text{Mag}}, \quad (2)$$

where θ is the angle between the two diffraction orders, Mag is the magnification of the microscope, and L is the period of the grating. For a grating of 80 lines/mm and total magnification of 150, a separation distance d of 500 μm gives a shearing distance of 125 nm, about half of the resolution, which is typically used for our experiments.

We use Hilbert transform, similar to that used in diffraction phase microscopy, to retrieve the phase gradient [3]. Figure 1 and Eq. (1) show only lateral shearing in the x direction. When a checkerboard grating is used instead of a Ronchi grating, shearing along two orthogonal directions is obtained simultaneously [10]. In combination with a spiral phase integration algorithm, the sample phase $\phi(x, y)$ can be obtained [7]. This approach is more resistant to vibration noise compared to diffraction phase microscopy, because no spatial filtering is involved and

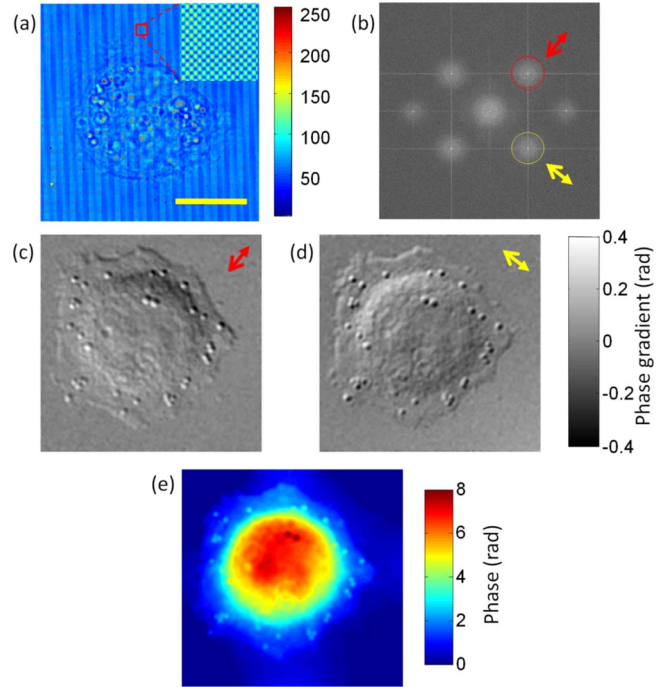


Fig. 2. (Color online) (a) Interferogram measured on the CCD (inset shows magnified fringes of the boxed area); (b) two-dimensional Fourier transform of the interferogram: the circles show the frequency component used for obtaining phase gradient images, while the arrows show the respective gradient directions; (c), (d) phase gradient images of the cell; (e) phase image of the cell obtained through spiral integration. Scale bar, 15 μm .

the use of a low-coherence source also significantly reduces the speckle problem.

To demonstrate live cell imaging, we acquired a two-dimensional interferogram of a live HeLa cell [see Fig. 2(a)] sandwiched between two coverslips using a checkerboard grating with a pitch of 9 μm and an illumination NA of 0.05. Through Hilbert transform of the two well-separated frequency components in Fig. 2(b) (indicated by circles), the amplitude and gradient phase images of the cell along both the diagonal and the anti-diagonal directions were obtained simultaneously. The two amplitude images show the exact same features (not shown), while the phase images show the phase gradient along two orthogonal directions [Figs. 2(c) and 2(d)]. Individual granules inside the cell are clearly visualized in the phase gradient images, although they are not observable in the amplitude images. We note that the phase gradient images are a special case of DIC. The illumination NA is kept very low so that plane-wave projection approximation can be used here, which facilitates direct integration to obtain the absolute phase of the entire cell. Figure 2(e) shows the integrated phase image. The features observed in DIC images are well preserved in the quantitative phase image. The quality of the phase image is much improved compared to laser-based quantitative phase imaging due to removal of speckle. Some residual phase variation in the background is likely due to the aberration present in the $4f$ imaging system.

Narrow optical sectioning, one of the most useful features in conventional DIC microscopy, can also be obtained in our system by increasing the illumination NA.

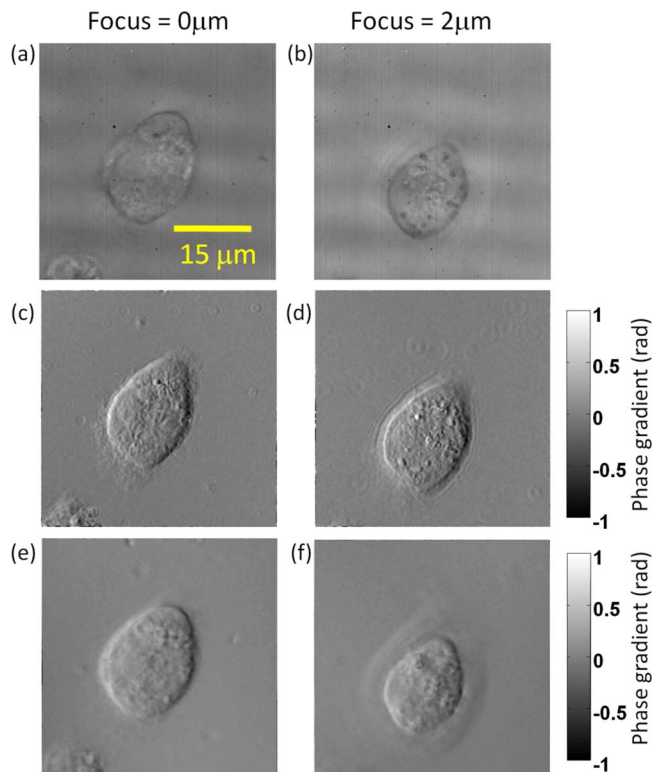


Fig. 3. (Color online) (a), (b) Bright-field images of a HeLa cell at different focal positions; (c), (d) phase gradient images of the cell under illumination NA of 0.05; (e), (f) phase gradient images of the cell under illumination NA of 0.8.

In our setup, it is easily achieved by opening the iris diaphragm of the condenser. In this case, the intensity S in Eq. (1) will be an integration of the entire illumination NA. Because of the destructive interference of light arriving from the out-of-focus plane, the phase image obtained will have an optical sectioning effect, similar to bright-field microscopy or conventional DIC microscopy. Figure 3 shows the difference between low-NA and high-NA illumination for another HeLa cell at different focal positions $2 \mu\text{m}$ apart. For comparison, bright-field images [Figs. 3(a) and 3(b)] are acquired using the same setup by blocking the first-order beam. At low illumination NA of 0.05, a diffraction ring pattern around small dust particles can be clearly observed in Figs. 3(c) and 3(d). However, at high NA of 0.8, the sample is illuminated by a superposition of plane waves of many different angles; the corresponding phase gradients add up incoherently,

suppressing the diffraction effect, as well as providing optical sectioning. Because of the angular average effect, the phase gradient under high-NA illumination is smaller than that for the low-NA illumination case. Unlike conventional DIC microscopy, increasing illumination NA for DIC based on lateral shearing beyond a certain point will decrease signal to noise quickly due to decreasing interferogram contrast [10]. This will limit the resolution obtainable with the off-axis DIC method. Nonetheless, we have shown that, up to the illumination NA of 0.8, the image contrast is still well maintained.

In conclusion, we have demonstrated a new (to our knowledge) approach to obtain quantitative DIC images of living cells. It has the advantage of simple and straightforward implementation on conventional microscopes at low cost, yet provides high-quality quantitative phase information to interrogate the structure and function of unstained living cells. Other modes of imaging, such as bright field and fluorescence, can be easily integrated without changing optical components. Toggling between optical sectioning and phase integration is easily controlled by adjusting the iris of the condenser lens. Quantitative phase can be obtained through spiral integration of the linear phase gradient images along orthogonal directions under low NA illumination.

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