Transport-of-intensity approach to differential interference contrast (TI-DIC) microscopy for quantitative phase imaging

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Differential interference contrast (DIC) microscopy is an inherently qualitative phase-imaging technique. What is obtained is an image with mixed phase-gradient and amplitude information rather than a true linear mapping of actual optical path length (OPL) differences. Here we investigate an approach that combines the transport-of-intensity equation (TIE) with DIC microscopy, thus improving direct visual observation. There is little hardware modification and the computation is noniterative. Numerically solving for the propagation of light in a series of through-focus DIC images allows linear phase information in a single slice to be completely determined and restored from DIC intensity values. © 2010 Optical Society of America

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Studying behaviors of cells and organisms in their natural living state is an important area in biological and biomedical research. However, without staining, these specimens are rarely visible under the conventional light microscope, as only phase shifts are introduced when light passes through such phase objects. The commercially available differential interference contrast (DIC) microscope is often utilized to enhance the contrast in this situation. Compared to some other phase-imaging techniques, DIC owes its popularity to higher transverse resolution owing to a partially coherent source, better depth discrimination, and the pseudo-3D relief type of image that is clean of artifacts [1]. However, one disadvantage is that the resulting image cannot be used directly for quantitative analysis, as the intensity is not linearly proportional to the phase distribution [2].

Motivated by the possibility of making DIC an even more versatile tool in biological laboratories, the above limitation has triggered much recent interest in techniques for obtaining a linear relationship to optical path length (OPL) from DIC images. Cogswell, Sheppard, and coworkers proposed an image formation theory in DIC [2] using a phase-gradient transfer function theory and subsequently a Fourier integration method [3] that requires phase-gradient information along two orthogonal directions, i.e., rotation of the object or rotation of the shear direction. Also, using phase-stepping algorithms to retrieve the phase-gradient information in DIC, Shribak proposed sample rotation-free algorithms to reconstruct both the linear OPL and birefringence distribution [4]. Finally, using iterative computing, Preza [5] and Biggs [6] individually proposed deconvolution processes for phase restoration from DIC images.

To avoid either significant hardware modification or complicated computation, we propose here a robust and noniterative approach that combines DIC with the TIE, and we name the technique TI-DIC. Originating from the free-space Helmholtz wave equation, TIE under the paraxial approximation outlines an elliptical second-order partial differential equation that recovers phase from a through-focus series of image intensities [7]. Consider a complex field, \( \sqrt{I(\vec{r},z)} \exp(i\phi(\vec{r},z)) \), immediately after the object; the phase of the field can be retrieved through

\[
\nabla_{\perp} \cdot [I(\vec{r},z)\nabla \phi(\vec{r},z)] = k \frac{\partial I(\vec{r},z)}{\partial z},
\]

where \( k \) is the wavenumber, \( (\vec{r},z) \) denotes the paraxial coordinate system, and \( \nabla_{\perp} \) is the 2D position in the transverse plane normal to the optical axis \( z \). The terms \( I(\vec{r},z) \), \( \phi(\vec{r},z) \), and \( \partial I(\vec{r},z)/\partial z \) denote the in-focus image intensity, phase to be retrieved, and longitudinal derivative of the intensity, respectively. The 2D gradient operator \( \nabla_{\perp} \) operates on the transverse plane only. The fact that TIE is not restricted to the coherent regime and works also with a partially coherent source [8] makes it applicable to a
wide range of experiments in the optical [9] and x-ray regimes [10] as well as in electron-beam microscopy [11]. This makes the TIE formalism valid with phase reconstruction in DIC images.

Not affecting the general result, a quantitative DIC model in the 2D coherent imaging regime can be set up for more intuitive understanding of the phase reconstruction process. Consider first the two sheared wavefronts,

\[ U_A = a_A(x, y) \exp(i\phi_A(x + \Delta x, y) - \theta), \]

\[ U_B = a_B(x, y) \exp(i\phi_B(x - \Delta x, y) + \theta). \]

(2)

The DIC image intensity can be modeled as

\[ I = |a_A|^2 + |a_B|^2 - 2a_Aa_B \cos(\phi_A - \phi_B - 2\theta). \]

(3)

Let \( |a_A|^2 \) and \( |a_B|^2 \) be the displaced intensity \( I_A \) and \( I_B \) from shear respectively, then \( I_A \) and \( I_B \) each satisfy Eq. (1). Substituting these into Eq. (3), we have

\[ \frac{\partial I}{\partial z} = \nabla \cdot (I_A \nabla \phi_A) + \nabla \cdot (I_B \nabla \phi_B) \]

\[ - \frac{2k}{\partial z} \sqrt{I_A I_B} \cos(\phi_A - \phi_B - 2\theta), \]

(4)

where the phase-gradient change along the axial direction is assumed to be small. This is generally true for thin biological samples. Given uniform illumination and no dichroism, i.e., \( I_A = I_B = I_0 \), let \( \phi_A = \phi_0 + \delta\phi \), \( \phi_B = \phi_0 - \delta\phi \), and the phase difference \( \delta\phi \) be small, Eq. (4) can be simplified to

\[ \frac{\partial I}{\partial z} = 2I_0 (1 - \cos(2\delta\phi - 2\theta)) \nabla^2 \phi_0. \]

(5)

Notice that the right-hand side of Eq. (5) is \( \nabla^2 \phi_0 \) multiplied by a scaled version of the original DIC image [Eq. (3)]. Thus after dividing both sides by in-focus DIC image intensity, a Fourier inverse Laplacian can be applied to solve Eq. (5) for \( \phi_0 \) with regularization treatment at zero spatial frequency. The reconstruction is independent of a bias value \( \theta \); however, a suitable \( \theta \) value will give better signal-to-noise ratio in the reconstruction. During the reconstruction process the image noise is amplified by the inverse of defocus distance \( \Delta z^{-1} \) [9]. However, increasing \( \Delta z \) will also decrease the accuracy of the linearity assumption that underlies our finite-difference derivative approximation. A program has been written to have a quick estimation of the noise profile in order to find the best possible \( \Delta z \) distance for each sample before reconstruction.

To test the capacity of the technique, we first imaged an unstained cheek cell with an Olympus 40×, 0.9 NA air objective using an Olympus BX61 DIC microscope under Köhler illumination with a DP30BW camera. The condenser aperture was set to be 70% of the objectives to optimize the coherence parameter \( S \) (NA condenser/NA objective) [12] for TIE. Two bias values \( \theta \) equal to \( \pi/4 \) and \( 3\pi/4 \) were chosen for a linear dependence region of DIC [2]. The defocus value is 1.5 \( \mu \)m from the best-focus position (Fig. 1, left column). Perhaps surprisingly, the two DIC images generate almost identical reconstruction images, despite having opposite bias. Actually this follows directly from Eq. (5) where the same \( \phi_0 \) is reconstructed. In fact, phase-gradient information that is embedded in a single DIC image is preserved in the proposed algorithm, and we verified this by comparing the phase-gradient information obtained from our method with that of a conventional method using phase-shifting DIC [13]. Both approaches retrieve the same phase-gradient information, as shown in Fig. 2, even though they are based on different data sets. This supports the view that there is no information lost in the proposed technique, and the reconstruction is valid and accurate.

To further demonstrate our method quantitatively, we imaged a well-characterized single-mode optical fiber (Corning Inc., SMF28) in air with the same experimental setup using a 20×, 0.75 NA objective with Köhler illumination. The protective coating of the fiber was striped. Three images at bias values of \( \pi/4 \) were taken with a defocus step of 1.5 \( \mu \)m. The recon-
conducted image shows the core of the fiber [Fig. 3(a)] dimly as the change in phase is very small compared with the total phase change but can be clearly seen after histogram adjustment [Fig. 3(b)]. To characterize the fiber, we validated that the refractive index difference between the core and the cladding agreed with theoretical values in the datasheet [see Fig. 3(c)]. We calculated theoretically the OPL profile of the cladding without the refractive index difference in the core region according to the given dimensions [Fig. 3(c)]. Then we fitted the experimental profile [across the line in Fig. 3(a)] excluding the points covering the core region and the regions outside the cladding to the theoretical value. The maximum OPL difference [value of \( \Delta \) in Eq. (5)] along the center of the core (after normalization) is found to be 0.32%, which is very close to the manufactured information of 0.36%, demonstrating that our method is able to detect minute phase changes quantitatively.

In summary, the technique presented herein provides the conventional DIC microscope with a powerful additional function without significant alterations to the setup. The proposed approach is direct and Fourier-computation based; thus quantitative phase information can be retrieved nearly in real time. Live cellular quantitative phase imaging has been obtained with TIE bright field reconstructions (TI-BF) [9]. Under the assumption that phase-gradient information is z independent (true for most thin biological cells), the recovered phase information from TI-DIC is the same as one obtains from TI-BF [Eq. (5)]. The current technique extends TIE to wider usages because phase contrast mechanism is vital in visually dealing with transparent and colorless samples in biological and medical research. The advantage of introducing TIE to DIC is immediate visualization and reconstruction. This greatly enhances the feasibility of real-time observations and experiments. See Fig. 4 (Media 1) for a sequential reconstruction video of macrophage cells (mouse macrophage-like cell line RAW 264.7) from DIC images acquired in a time series. The prospect of real-time live-cell quantitative phase imaging is interesting in that a cell’s dry mass is linked to its protein content, opening up an interesting regime of new applications in biomedical research.

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References