

METHODS FOR VISUALIZATION , PHASE RECONSTRUCTION, AND DECONVOLUTION IN 3D DIFFERENTIAL INTERFERENCE CONTRAST MICROSCOPY

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Nomarski Differential-Interference-Contrast (DIC) microscopy is widely used to study structural features of live biological specimens. Among the advantages of DIC is the ability to reveal contrast generated by local differences in the optical path length (OPL) throughout the specimen; features otherwise invisible in conventional optical microscopy. The technique uses two pairs of Nomarski prisms. The image is created by interference between two orthogonally polarized wavefronts separated spatially in what is called the shear direction of the first prism. The second prism recombines the two wavefronts after they pass through the specimen and interference occurs at the analyzer polarizer. The resulting image reveals the characteristic DIC ‘bas-relief’ display of the OPL; effectively a directional derivative of the OPL in the specimen, combined with contrast caused by the absorption of light by the specimen. The contrast, and relative intensity, of the phase and amplitude components of the specimen, are controlled by the degree of polarization extinction, denoted the bias. The DIC image also gives good rejection of light from out of focus features and good resolution of fine structures. Unfortunately, light absorption from the specimen, the ‘bas-relief’ image character, and aspects of the bias setting can make images difficult to interpret and hinder the application of image processing methods, such as deconvolution. The nature of the image also makes it especially difficult to visualize 3D structures when optically sectioned images are taken, even though the z-axis resolution is excellent with DIC.

This situation has been addressed by many prior workers mostly by using methods to reconstruct the OPL, or phase image, of the specimen. These methods include line integration [1], deconvolution [2], and rotation of the specimen, the shear, and/or bias [3,4]. Although no satisfactory method has been devised to perform a full phase reconstruction from a single practical image (with phase and absorption included), many of these methods have utility in the improvement of specimen structure visibility. There has also been success using the Hilbert transform to effectively conceal the DIC nature, yet retain sharp image detail and permit 3D visualization.[5] We will review the attributes of these various methods to improve the practical utility of the DIC technique when coupled with opportunities made possible using modern CCD imaging and image deconvolution.

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