

**ARE THE COMMON METHODS OF PHASE RETRIEVAL FROM DIC IMAGES  
SUFFICIENTLY COMPLEX?**

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Differential interference contrast microscopy (DIC) is mainly used as a popular method for imaging optical path length gradients in unstained objects, such as, e.g., biological specimens. Several approaches have been published, which have attempted to convert observed image intensities to underlying path length differences, e.g., [1-3]. At FOM 2005, we presented a theoretical analysis of the origins of contrast in DIC imaging [4], which has revealed the role of variations in bias retardation. Such variations are due to the refraction of transmitted illumination light (as caused by any part of the object that exhibit gradients of optical thickness), which gives rise to an object-detail-specific deflection of condenser aperture image in the objective back focal plane. Unfortunately, we have failed several times to devise an appropriate model that would support the above notion with convincing experimental data. However, we found out recently how to demonstrate clearly that the refraction-induced changes of bias retardation control actually the image brightness and appearance in DIC microscopy to a much larger extent than the plain phase shifts due to the optical gradients can do (J. Microsc., manuscript in preparation). This proof involves: i) the assessment of phase shifts that control the actual brightness in the images of calibrated glass wedges, followed by the comparison of measured data with the estimated values of respective phase difference contributions due to the optical thickness gradients, and the refraction-induced changes of bias retardation, ii) the measurement of brightness profiles in the DIC images of glass semi-cylinders immersed in media of different refraction index. Our finding that the actual image brightness in DIC images is mainly controlled by the refraction-induced deflection of partial images of condenser aperture in the objective back focal plane has also the following interesting corollary: It indicates that the major optical effect behind the performance of DIC microscopy is in fact identical with that employed in the Hoffman modulation contrast (HMC), which is the refraction of transmitted illumination light by object details. From this point of view, the main difference between HMC and DIC seems to be the way how the angular deviation of refracted rays is converted into the brightness of a particular image detail. Instead of detecting the deflection of condenser aperture image with a specific modulator placed in the objective back focal plane, as used with HMC, it is the aperture-image-deflection dependent change in bias retardation, which makes DIC more sensitive to moderate optical gradients than HMC.

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