

## PROBLEMS IN QUANTITATIVE DIC MICROSCOPY

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Differential interference contrast microscopy (DIC) is mainly used as a popular method for imaging optical pathlength gradients in unstained objects, such as, e.g., biological specimens. Several approaches were published, which have attempted to convert observed image intensities to underlying pathlength differences, e.g., [1,2]. Unfortunately, the feasibility of various quantitative approaches to the evaluation of DIC images was often demonstrated with computer-generated simulations of objects and their DIC images, e.g., [3,4]. In all the quantitative models of DIC imaging it was tacitly assumed that the apparent value of DIC bias retardation (which can be varied by translating one of the Wollaston prisms along the shear direction) is not correlated with the object properties. It will be demonstrated in this contribution that for real objects the actual values of bias retardation, and thus the resultant brightness in their DIC images can be significantly influenced by the optical phenomena directly related to the occurrence of optical pathlength gradients, like refraction, reflection, and diffraction of illumination light. The optical phenomena listed above deviates the rays of illumination light, and thus shifts the site of their incidence at the objective Wollaston prism, which results inevitably in the object-dependent variations of bias retardation.

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