A HIGH NUMERICAL APERTURE, VECTORIAL THEORY OF CONFOCAL, SCANNING DIFFERENTIAL INTERFERENCE CONTRAST MICROSCOPY.

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KEY WORDS: high aperture theory, numerical simulations, differential interference contrast, Nomarski.

1 INTRODUCTION

Transmitted light differential interference (DIC) microscopy is used widely for imaging phase objects such as unstained biological specimens. It is also used in reflection mode for measuring surface topography in fields such as materials science.

Gailbraith introduced the first imaging model of a wide field DIC microscope [1] which calculated the image of a bright spot for various imaging parameters. Subsequent models, e.g. Holmes and Levy [2], were proposed which allowed analysis of more general phase objects. Later, Cogswell and Sheppard [3] developed a confocal DIC and demonstrated that it had superior optical sectioning to that of a non-confocal DIC. They also developed a rudimentary imaging model using a transfer function approach. This model was useful for demonstrating that the advantage of a confocal DIC microscope is that it rejects out of focus, low frequency components of scattered light.

This and previous models are based upon scalar theory. It is well known that when high numerical aperture (NA) objectives are employed in conventional confocal microscopes, the polarisation of both focussed incident and scattered light must be considered. This is also true for confocal DIC microscopes. We have constructed a high NA, vectorial model of confocal DIC microscopy. Our presentation will demonstrate the theory behind this model as well as the results which may be obtained using it.

2 THE MODEL

The model employs high NA theory to calculate the electromagnetic field of the focussed beam that has propagated through a Wollaston prism. We rigorously calculate how the incident illumination is scattered by the sample. Complex specimens require the use of a rigorous numerical techniques such as the finite difference time domain method (FDTD). Scattering by simpler objects such as points or planes may be calculated using analytic solutions to Maxwell's equations.

To illustrate the model, the image of a point object in reflection has been calculated for various bias values. An 100×0.85NA objective was employed with a horizontal sheer (separation of focussed spots) of 190 nm. The images are shown in Figure 1.

Our model is useful in both biological and tomography applications. It is able to model objects of greater complexity that previous models.

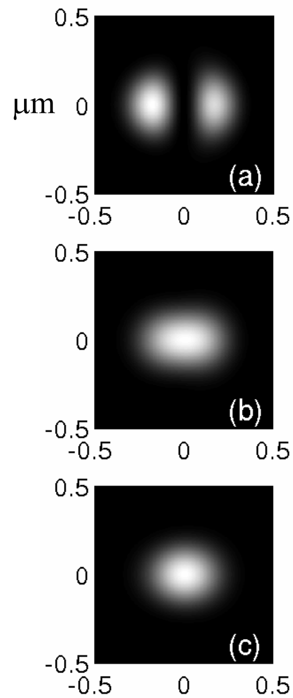

Figure 1: DIC image of a bright spot for bias of 0 (a), π/2 (b) and π(c).

[1] T.J. Holmes and W.J. Levy, "Signal-processing characteristics of differential-interference-contrast microscopy," *Applied Optics* 26, 3929-3939 (1987).
[2] W. Gailbraith, "The image of a point of light in differential interference contrast microscopy: Computer simulation," *Microsc. Acta* 85, 233-254 (1982).
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