

THREE-DIMENSIONAL QUANTITATIVE PHASE IMAGING IN DIC (DIFFERENTIAL INTERFERENCE CONTRAST) MICROSCOPY

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Phase contrast imaging is a specific technique in optical microscopy that is able to capture the minute structures of unlabeled biological sample from contrast generated in the variations of the object's refractive index. It is especially suitable for living cells and organisms that are hardly visible under conventional light microscopy as they barely alter the intensity and only introduce phase shifts in transmitted light. Optical phase imaging has great potential in biomedical applications from examining both topological and three-dimensional biophysical properties of cells and organisms (Fig. 1).

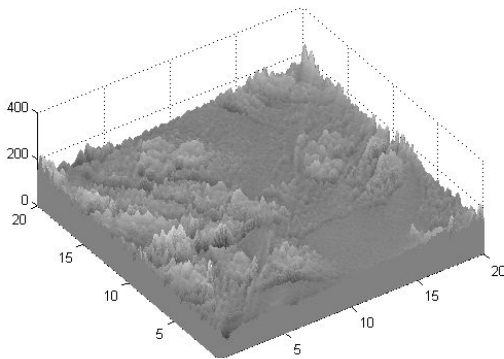


Fig. 1 Surface profiling of cells with quantitative phase imaging

Conventional DIC microscopy with partially coherent light source is a very powerful technique for phase imaging, and is able to yield higher lateral resolution compared to other interferometric phase imaging methods [1]. However, it is inherently qualitative and the information obtained is a phase-gradient image rather than a true linear mapping of OPL differences. We propose a novel method here that extends the Hilbert transform and later combines the correlation of light intensity and phase

with polarization-modulated differential interference contrast (DIC) microscopy. Numerically solving the relationship of light propagation in a series of through-focus DIC images allows linear phase information to be completely determined and restored from phase gradients in two-dimensional planes. Quantitative phase information in three-dimensional space can then be reconstructed from 3D rendering of the calculated phase images. Initial results of application in biological cells are also demonstrated.

REFERENCE:

[1]. C. J. Cosgwell and C. J. R. Sheppard, "Confocal differential interference contrast (DIC) microscopy: including a theoretical analysis of conventional and confocal DIC imaging", *J. Microsc.*, **105**, 81-101, (1992).