IMAGING THREE-DIMENSIONAL REFRACTIVE INDEX DISTRIBUTION WITH DIFFERENTIAL INTERFERENCE CONTRAST (DIC) MICROSCOPY

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Differential interference contrast (DIC) microscopy is well known for its ability to image transparent phase objects that otherwise produce very little contrast in conventional brightfield microscopy. DIC microscope records the interference fringe produced by two sheared wave fronts passing through the common specimen. As a result, there is a nonlinear relationship between image intensity and the magnitude and phase gradient of the object. Significant progress has been made recently in extracting quantitative phase information from the qualitative intensity map recorded by a DIC microscope, such as phase shifting DIC microscopy [1] and the approach based on the transport of the intensity equation (TI-DIC) [2]. Most quantitative phase imaging work is, however, restricted to a thin specimen treated as a 2D planar object. For thick objects, tomographic approach similar to CT has been proposed to obtain a three dimensional distribution of refractive index [3]. The main drawback of the CT-like approach is the requirement of a 3D rotation of the specimen or the probing beam which is difficult to implement in a routine microscope.

In this study, we first present the transport of the intensity equation for a 3D thick object which serves as a natural extension to TI-DIC for planar objects derived in [2]. We then outline an algorithm to obtain three dimensional refractive index map for a 3D phase object from a series of images recorded by a DIC microscope at a set of different focus positions (z-stack). The performance of the algorithm is assessed by monitoring the temporal evolution of apoptosis and necrosis of human breast MCF7 epithelial cells. The proposed approach may enable conventional DIC microscope imaging 3D refractive index distribution in living cells with minimal alteration.

References: