Making Quantitative DIC Microscopy a Reality

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Experimental verification of our previously proposed linear phase imaging technique for differential interference contrast microscopy (DIC) microscopy will be presented. This technique first applies phase-shifting methods to DIC to acquire linear *phase gradient* images in two orthogonal directions. A special Fourier integration algorithm is then applied to the combined *phase gradient* images to create a single linear *phase* image in which intensity is proportional to phase. This overcomes the limitations of traditional DIC, which cannot accurately measure the phase (i.e. refractive index or thickness) of embedded 3D phase objects.

DIC microscopy is well known for its ability to image transparent phase objects that otherwise produce very little contrast in bright-field microscopy. Its particular advantages over other phase imaging techniques include: applicability at high numerical apertures, ability to image phase objects embedded within a transparent material, and lack of aberrations. Until recently, one disadvantage of DIC was its non-linear intensity versus spatial frequency response. In other words, intensity of a DIC image was not linearly proportional to the differential phase of the object. In 1997 Cogswell et al. showed that geometric phase-shifting techniques could be applied to DIC to remove this non-linearity.[1]

Theoretically, integration of images created using this technique leads to final images in which intensity is linearly proportional to the true phase of the object. Therefore, phase-shifting DIC (PS-DIC) seems an ideal starting point for measurement of 3D phase objects. However, previous attempts to integrate PS-DIC images using numerical integration produced inadequate phase image results. These images showed directional artifacts due to the unknown constant of integration.[2]

A newly proposed method of integration has shown promising results in simulation.[3] Based on a Fourier filtering technique, this method has been dubbed *spiral phase integration* due to the spiral nature of the filtering function's phase angle. The symmetry of the spiral phase filtering function prevents the directional artifacts seen in numerical integration. Intensity of phase images created using this technique in simulation is linear with the phase of the object, if one allows for some error off-axis and at image and object edges. In this paper, we show recent experimental results that address these errors and further approach our goal of producing a calibrated DIC system where image intensity can be directly correlated to the thickness or refractive index of the specimen features.

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