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Computational Model of DIC Microscopy for Reconstructing Specimens

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Abstract. **Biologists often use Differential Interference Contrast (DIC) microscopy to study live cells. However, they are limited to qualitative observations due to the inherent nonlinear relation between the object properties and image intensity. As a first step towards quantitatively measuring optical properties of objects from DIC images, we develop and verify a computational model for the image formation process. Next, we plan to use this model to reconstruct the properties of unknown specimens.**

Keywords: Optical microscopy, light-propagation model, polarization ray tracing.

1 Introduction

The Nomarski DIC microscope is the preferred method for visualizing live biological specimens. The nonlinear relation between object and image intensity limits biologists to qualitative analysis. In this paper, we describe a computational model of the DIC imaging process. The model is the first step in quantitatively reconstructing properties of the specimen. We verify the model by comparing real data from known specimens with simulated images of the corresponding virtual objects.

Looking through the eyepiece, in a DIC microscope, an observer sees an approximate differential of the object's phase function. The differential is along a particular (shear) direction, in the transverse plane. In the microscope, a birefringent prism shears an incident wavefront. The sheared waves propagate a differential distance apart through the specimen. The image intensity is the interference pattern of the waves. Out-of-focus amplitude contributions further obscure the image.

2 Computational Model

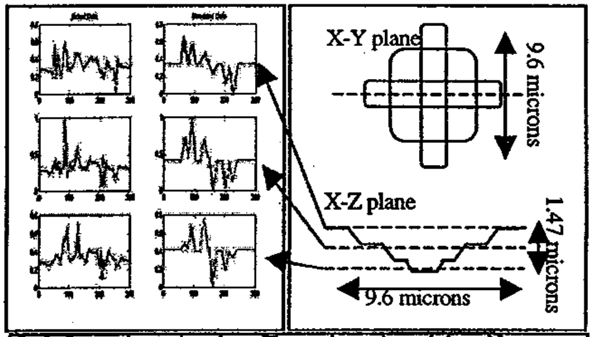
Our computational model consists of a ray tracer and an approximation of the wave aberrations due to diffraction by the lens and due to the self-occlusion by the object. We use polarization ray-tracing methods to trace rays through the prisms. The object is a grid of voxels, each assigned a refractive index value. We extract light paths, propagating through the object, which minimize Fermat's variational integral.

Three-dimensional object structure can be recovered by modeling the wave propagation from different planes in the object. To correctly model in- and out-of-focus contributions, we develop an aberration model. The shape of the wavefront reaching the lens from each point in the object is approximated. After numerous simulations, we determine that the most significant aberrations can be approximated as defocusing effects. The image intensity is calculated as the contribution of these aberrated wavefronts diffracted by the lens.

3 Results

First, we experimentally calibrate the bias between the two polarized wavefronts, introduced by the prism. We image an interference pattern due to two polarized, planar wavefronts propagating through the prism. The calibration is based on analysis of this image.

Next, to validate our model, we ion-milled a pattern onto a glass wafer (Fig. 1). The index of refraction for the glass is 1.53. The cavity was filled with oil of refractive index 1.45. We compare intensity plots (fig. 1) from the real images and simulated images of the wafer. Though only intensity plots are shown, the simulations consist of an optically sectioned, three-dimensional set of images. Top to bottom, .5 microns separate the images axially. In both the simulated and real images, the correct features are in focus at the corresponding. The consistent correspondence between the out-of-focus and in-focus features is crucial to the reconstruction task.



Left: Image intensity plots. First column is real data. Next column is corresponding simulated images. Right: Wafer schematic. Dashed lines correspond to intensity plot locations.

4 Reconstruction Results

We have conducted 1-d simulation experiments illustrating a reconstruction method. We use our model in an iterative nonlinear optimization method. Experiments with real, higher-dimensional data are underway.

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