Quantitative phase imaging of biological structures with computational phase-shifted differential interference contrast microscopy

King, S. V. , [1] Libertun, A. R., [1] Piestun, R.,[1] Preza, C [3]and. Cogswell, C. J.[1,2]
[1]Department of Electrical and Computer Engineering, Campus Box 425,
University of Colorado, Boulder, CO 80309-0425, sharon.king@colorado.edu
[2] CDM Optics, Boulder, CO 80303

[3]Department of Electrical and Computer Engineering, 206 Engineering Science Bldg,
The University of Memphis, Memphis, TN 38152-3180,cpreza@memphis.edu

Digital signal processing of phase-shifted Nomarski differential interference contrast microscopy (DIC) images from two directions of shear enables isotropic linear phase imaging with high resolution and high contrast.

Using a spiral phase pseudo-inverse filter, we apply a Fourier domain integration technique to simulated and experimentally acquired, phase-shifted DIC images of partially absorptive test objects. Linear regression analysis of phase imaging results from known objects is used to calibrate image intensity values to phase values. Analysis of these phase imaging results shows image values can be calibrated to provide accurate phase measurements of objects regardless of their symmetry or absorption properties even when embedded in optically transparent media. Analysis of simulated results from imaging of phantom bead test objects, with phase values in the range of 0-1 radians, predicts a calibration accuracy of ±0.06 radians (λ/110). Analysis of experimental results from imaging of real test objects, with phase values in the same range, shows a larger calibration error interval. Experimental results from 64x, 1.4 NA images of BPAE cultured cells demonstrate the possibilities for quantitative phase imaging of finely detailed biological structures using this method.