QUANTITATIVE DIFFERENTIAL INTERFERENCE CONTRAST MICROSCOPE WITH SWITCHABLE SHEAR DIRECTION

Michael Shribak
Marine Biological Laboratory, 7 MBL Street, Woods Hole, MA 02543, USA
E-mail: mshribak@gmail.com

KEY WORDS: Differential interference contrast, DIC, phase, microscopy, live cell imaging

A conventional differential interference contrast (DIC) microscope is based on the interference of two beams, which optic axes are sheared by a sub-resolution distance. When the beams are traveling through the specimen the interference creates image contrast, which depends on the gradient of optical path difference (OPD) encountered along the shear. The contrast disappears if the OPD gradient is perpendicular to the shear direction. The image contrast also depends on the bias OPD introduced by microscope and specimen's absorption and/or scattering. The DIC image is not quantitative.

The orientation-independent (OI-) DIC microscope overcomes these limitations. The OI-DIC rapidly switches the shear direction by 90° and controls the bias. The microscope captures two complementary sets of DIC images with orthogonal shear directions. The images are processed to compute OPD map, which displays the specimen's morphology and can serve as a landmark for fluorescent staining. The OPD map can be used to measure the sold content (DNA, protein) of the cells and to reconstruct the refractive index.

Other interference and phase microscopy techniques use modified or restricted numerical apertures (NA) of the condenser and/or objective lens. Often times the contrast of their raw images is low and is strongly affected by wavefront aberrations. The OI-DIC employs: (1) the full NAs of condenser and objective lenses, (2) high-contrast raw images, (3) the optical image subtraction, and (4) the computation image subtraction. Thus, the OI-DIC can provide the best resolution images.

We have confirmed that a standard research grade light microscope equipped with the OI-DIC and 100x/1.3NA objective lens, which was not specially selected for minimum wavefront and polarization aberrations, provides OPL noise level ~0.5 nm and lateral resolution ~260 nm at a wavelength of 546 nm. The new technology is the next step in development of the DIC microscopy. It can replace standard DIC prisms on existing commercial microscope systems without modification. This will allow biological researchers that already have microscopy setups to expand the performance of their systems.

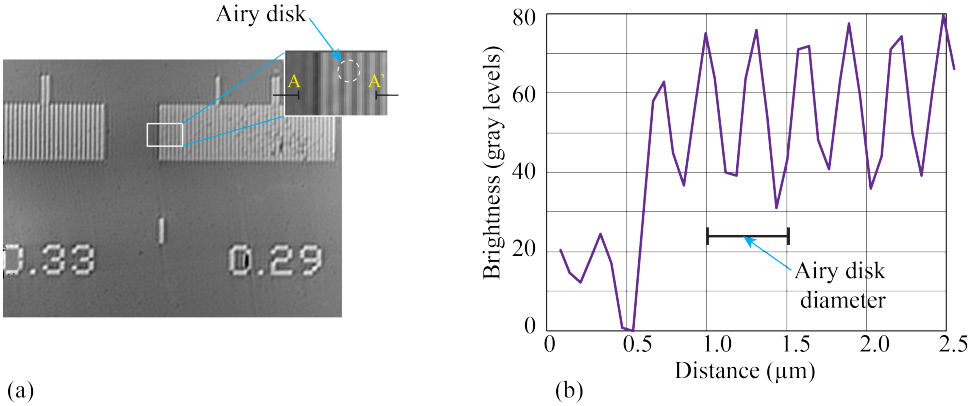

Figure 1: (a) Image of phase grating with step 290 nm computed with using deconvolution; (b) Cross-sections A-A' of the image brightness.

[1] M. Shribak, K. Larkin, D. Biggs, "Mapping of optical path length and image enhancement using orientation-independent differential interference contrast microscopy", *Journal of Biomedical Optics*, vol. **22**, No.1, 16006 (2017).