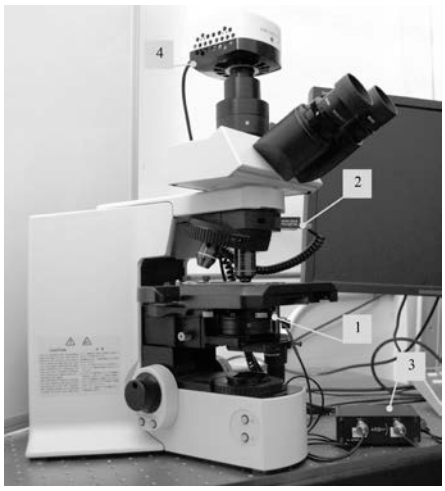
Quantitative orientation-independent differential interference contrast (OI-DIC) microscope

The conventional differential interference contrast (DIC) microscope shows the two-dimensional distribution of the optical phase gradient encountered along the shear direction between two interfering beams. Therefore, the contrast of DIC images varies with the orientation of the phase gradient with respect to the shear direction, giving DIC images the characteristic relief appearance. The image contrast also depends on the initial phase difference (bias) between the interfering beams. The OI-DIC microscope allows the bias to be modulated and shear directions to be switched rapidly without mechanically rotating the specimen or the prisms. A set of raw DIC images with orthogonal shear directions and different biases is captured within a second, which then used to compute the quantitative phase image.

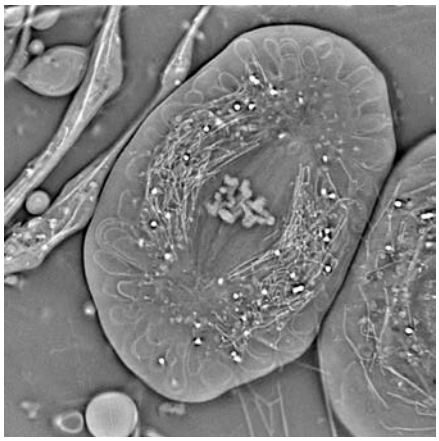
The new OI-DIC technique can be used with any objective lens at full aperture and provides an optical path length (OPL) or phase map at the highest resolution attainable with the imaging optics. Combining fluorescence microscopy with the OI-DIC imaging provides the molecular specificity of fluorescence and quantification of OI-DIC. The type of organelles in a live specimen could be scored using fluorescence markers, and subsequent organelle development followed during a long time series using OI-DIC with minimal phototoxicity.

A standard research grade light microscope equipped with OI-DIC and 100x/1.3NA objective lens provides lateral resolution ~250 nm and a discrimination depth ~100 nm at a wavelength of 546 nm. To the best of our knowledge, other currently available interference and phase microscopy techniques cannot achieve these parameters.

The new OI-DIC can replace standard DIC prisms on existing commercial microscope systems without modification. The OI-DIC is ideal for studying structure and motion in unstained living cells and isolated organelles, because cells can be followed for long periods of time non-invasively. Examples of some specific applications include the study of mitosis and meiosis, organelle movement, morphogenesis, etc.



OI-DIC components installed on upright microscope Olympus BX61. (1) Condenser-side beam-shearing DIC assembly; (2) objective-side beam-shearing DIC assembly; (3) Arcoptix USB LC-cell controller; (4) Lumenera monochromatic CCD camera.



OI-DIC image of the crane fly spermatocyte (full metaphase of meiosis-I). The three autosomal bivalent chromosomes are pulled apart at the spindle equator, along with one of the X-Y sex univalents located on the right. The distribution of tubular mitochondria surrounding the spindle and granual chromosome structure is clearly visible.

Reference:
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).