QUANTITATIVE ORIENTATION-INDEPENDENT DIC MICROSCOPE WITH EXTERNAL OPTICAL PATH

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We report a new advance in differential interference contrast (DIC) microscopy. DIC is a two-beam shearing interferometric technique, which is widely used for biomedical applications. The image contrast in conventional DIC images shows a gradient of refractive index along the shear plane. But the contrast is not quantitative, and structures with refractive index gradients in the perpendicular direction are invisible. Therefore, the operator must rotate the specimen under investigation mechanically.

Recently, we developed a quantitative orientation-independent DIC (OI-DIC) microscope, which rapidly rotates the shear direction without introducing any mechanical movement [1, 2]. OI-DIC microscopy offers significant advantages compared to other currently available quantitative phase microscopy (QPM) techniques. It uses the unrestricted full numerical aperture (NA) of the illumination and imaging beam paths and therefore provides the best lateral and axial resolution, the lowest light energy loss and allows for the shortest exposure time. Because of wide-spectrum, non-coherent illumination, the image does not suffer from speckle noise and the user can choose a spectral range that is most suitable for the specimen.

To take full advantage of OI-DIC, combination with fluorescence imaging is mandatory. To avoid compromises in either of the two imaging modes, beam paths should be separated, which requires the birefringent OI-DIC optics in the detection beam path to be moved away from the objective lens. We built an OI-DIC setup featuring an external optical path. The OI-DIC optics is placed in a re-imaged conjugated plane outside of the microscope. This arrangement also makes it possible to move the beam-shearing assembly exactly into the re-imaged back focal plane and thereby create better OI-DIC images.

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[2] J. Malamy, and M. Shribak, “High resolution imaging of epithelial cell migration and wound healing in a Cnidarian model using an orientation-independent DIC microscope”, *Journal of Microscopy*, **270** (3), 290-301 (2018).