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doi:10.1017/S1431927622006225
Microsc. Microanal. 28 (Suppl 1), 2022

Inspired By Shinya Inoue: Quantitative Orientation-Independent Differential Interference Contrast Microscopy

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Differential interference contrast (DIC) microscopy is a two-beam shearing interferometric technique that is widely used for biomedical applications. The advancement of DIC techniques has a long history in the Marine Biological Laboratory (MBL). About fifty years ago two prominent MBL scientists Shinya Inoue and Bob Allen implemented new high sensitive microscopy techniques using videocameras. Shinya was mostly concentrating on development of the video-enhanced polarized light microscope (PLM) [1]. Bob's main efforts were devoted to video-enhanced DIC [2]. Both PLM and DIC techniques were greatly improved at the MBL. PLM and DIC are two complementary label-free techniques. While PLM generates an image of birefringence, the DIC technique produces a phase gradient image. For example, PLM is used for imaging spindles, and DIC is used for imaging chromosomes. But these techniques have the same shortcoming. Their contrast is not quantitative, and it is strongly dependent on the specimen orientation. The researcher has to mechanically rotate the specimen under investigation. The imaging takes a lot of time. In 1994 Rudolf Oldenbourg, who worked with Shinya, proposed the quantitative orientation-independent differential polarized light microscope (LC Pol-Scope) [3]. This new microscope could generate a quantitative map of birefringence within seconds. There was an apparent need to develop a similar DIC microscope. In 2002 Michael Shribak, together with Shinya, tested the first prototype of orientation-independent differential interference contrast (OI-DIC) on an advanced microscope, called the "ShinyaScope" [4-6].

The OI-DIC microscope rotates the shear direction rapidly and without any mechanical movements [1, 2]. This microscope offers significant advantages in comparison to other currently available quantitative phase microscopy (QPM) techniques. It uses unrestricted full numerical aperture (NA) of the illumination and imaging beams, and therefore provides the highest lateral and axial resolutions, has the lowest light energy lost and has the shortest exposure time. Because of wide-spectrum non-coherent illumination, the image does not suffer from speckle noise. The user can choose a spectral range that is most suitable for the specimen. The optical image subtraction of two slightly different wavefronts allows observation of deep layers in scattering specimen.

Unfortunately, OI-DIC microscopy is not sensitive to horizontal surfaces. Therefore, OI-DIC cannot determine the organelle thickness precisely. However, this can be accurately measured by a confocal microscope. A combination of OI-DIC and confocal microscopes creates a win-win situation. The combined microscope provides high-resolution maps of optical path difference (by OI-DIC) and organelle thickness (by confocal), which can be used to generate a 3D volume image of refractive index or protein concentration (dry mass) with chemical specificity. The OI-DIC can be also integrated with a super-resolution STED microscope. This will enable us to image the ER and contact site proteins by two STED fluorescence channels and simultaneously visualize mitochondria in OI-DIC.

A major problem with combining these techniques is that the DIC prism splits excitation laser scanning and emitted fluorescent beams by a small shear distance, which affects the resolution of confocal image.

Also, DIC prisms alter polarization of the laser scanning and fluorescent beams. In order to avoid these issues, we built an OI-DIC with an external optical path. The OI-DIC optics are placed in a re-imaged, conjugated plane outside of microscope, and fluorescent and DIC channels are separated by a filter before reaching these optics. We found that OI-DIC with external optical path works actually better then the previous OI-DIC because we can place the beam-shearing assembly exactly in the re-imaged back focal plane by moving the assembly along the beam axis. DIC prisms and objective lenses are made with some tolerance, and always there is a mismatch between the back focal plane of objective lens and the interference plane of DIC prism.

The OI-DIC can also be combined with super-resolution microscopy techniques, for example with STED. We are currently working on combining the OI-DIC with single-molecule localization microscopy and will report the obtained results

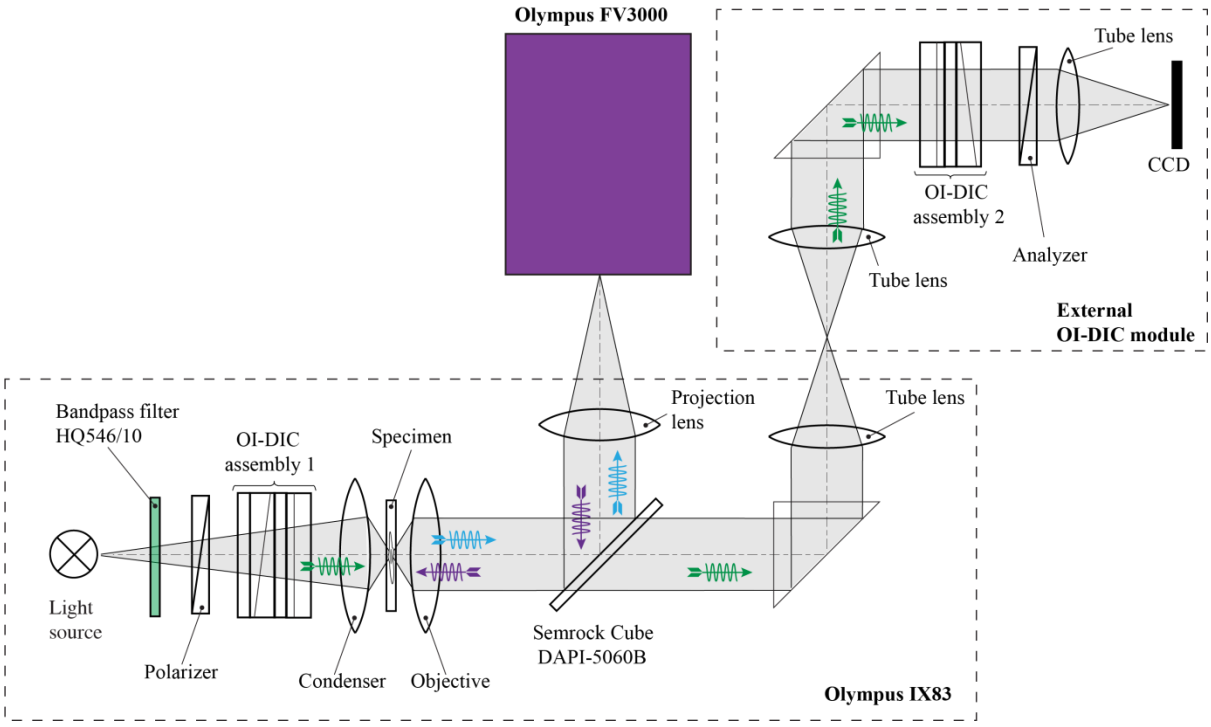

Figure 1. Schematic of microscope Olympus IX83 with external OI-DIC module and confocal module Olympus FV3000.

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https://doi.org/10.1017/S1431927622006225 Published online by Cambridge University Press