

SIMPLE DIC & PHASE IMAGING IN CONJUNCTION WITH CONFOCAL MICROSCOPY

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ABSTRACT

A simple method is described that allows the calculation of Normaski, differential interference contrast (DIC), phase, Zernike phase, darkfield or Hoffman modulation contrast images from a series of brightfield images, collected with the transmitted light detector (TLD) of a confocal laser scanning microscope (CLSM). The brightfield images may be collected simultaneously with confocal images. This method also allows the calculation of contrast-enhanced images from archival data. The current optical methods to collect DIC or Phase images with a TLD in conjunction with CLSM can be technically challenging and inefficient. The technique described here allows for the creation of contrast enhanced images such as DIC or Phase, without compromising the intensity or quality of confocal images collected simultaneously. Provided the confocal microscope is equipped with a motorised z-drive and a TLD, no hardware or optical modifications are required. The contrast-enhanced images are calculated with software using the Quantitative Phase-amplitude Microscopy (QPM) technique [1, 2]. This technique, being far simpler during image collection, allows the microscopist to concentrate on their confocal imaging, and experimental obligations. Unlike conventional DIC, this technique may be used to calculate DIC images when cells are imaged through plastic, and without the use of expensive strain free objective lenses. This technique may also be used to calculate phase, Zernike phase, darkfield and Hoffmann contrast images (Figure 1).

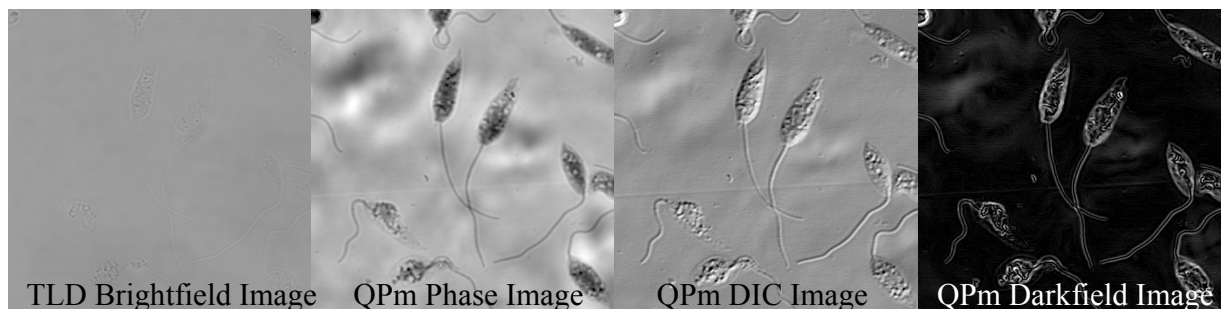


Figure 1: TLD brightfield image of *Leishmania Promastigotes* obtained from Leica CLSM, and computer generated QPm Phase, DIC and Darkfield images using three of the TLD brightfield images.

REFERENCE:

1. E.D. Barone-Nugent; A. Barty, and K.A. Nugent. "Quantitative phase amplitude microscopy I: optical microscopy." *J. Microsc.* **206**, 194-203 (2002).
2. www.iatia.com.au