Full-field phase-gradient contrast methods for label-free quantitative imaging of cellular morphology: AIDPC and DIC

Shalin B. Mehta[1,2,3] and Colin J. R. Seppard[1,2,4]

[1] Optical Bioimaging Lab, Div. of Bioengineering, National University of Singapore, Singapore 117574
[2]NUS Grad. School of Integrative Sciences & Engineering, National University of Singapore, Singapore
[3]Liver Cancer Functional Genomics Lab, Humphrey Oei Inst. Cancer Res., Natl. Cancer Ctr. Singapore
[4]Department of Biological Sciences, National University of Singapore, Singapore
Email: shalin@nus.edu.sg, shalin.mehta@gmail.com

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Label-free quantitative measurement of morphological features of cells (e.g., size of nucleus, thickness of cell, displacement of vesicles) requires optical processing approach that relates either the specimen's phase information or phase-gradient information linearly to the image. Phase imaging methods generally require coherent illumination, leading to moderate spatial resolution and serious coherent artifacts due to mottle. On the other hand, phase-gradient contrast can be achieved while using large illumination aperture (i.e., partially coherent illumination) leading to twice the resolution, depth sectioning and reduction in mottle as compared to coherent system. **Differential Interference Contrast** (DIC) is a currently popular phase-gradient contrast method. However, DIC images a complex mix of absorption and phase-gradient information. DIC relies on manipulation of polarization to achieve phase-gradient contrast and hence fails to image birefringent specimens and specimens grown on plastic. We have proposed a novel approach based on oblique illumination called **Asymmetric Illumination based Differential Phase Contrast** (AIDPC) that overcomes these limitations and allows good separation of phase-gradient and absorption information. In this presentation, DIC and AIDPC are compared in terms of image formation process, instrumentation, and experimental images. AIDPC lends itself to automation and simultaneous fluorescence imaging.

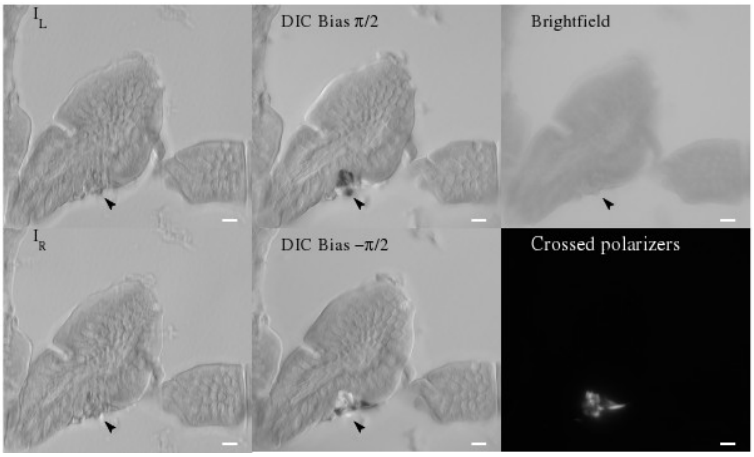

Figure 1: Images of Mouse Intestine section (Invitrogen Fluocells prepared slides # 4). I_L and I_R are images acquired using left and right semi-circular apertures and used for computing AIDPC image. Phase variations in birefringent regions (marked by arrow and appearing bright between crossed polarizers) are masked under DIC, but imaged with AIDPC. Scale bar is 10µm.

References:

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