Asymmetric illumination based differential phase contrast (AI-DPC) for full-field transmission imaging of phase information in biological specimens

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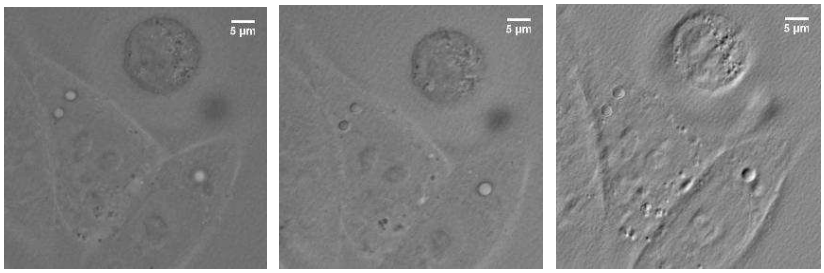
Differential phase contrast (DPC) is a simple yet powerful quantitative phase gradient imaging method which has been implemented using split-detector in laser scanning microscopes (1). The principle of reciprocity suggests that equivalent imaging can be performed with 'split-illumination' in the full-field transmission mode. In this presentation, we give the first demonstration of the validity of this hypothesis. We synthesize split illumination by subtracting two images (say A and B) obtained with oppositely placed half-aperture stops in condenser back focal plane using a CCD detector.

DPC has certain advantages over the popular phase gradient imaging method of differential interference contrast (DIC) while still producing a bas-relief appearance as does DIC. Its simpler image formation process facilitates easier retrieval of phase information independent of amplitude information from the ratio (A-B)/(A+B). It does not rely on manipulation of polarization and therefore works well with plastic substrates on which biological specimens are usually grown. It is more light efficient and hence less phototoxic to cells and is much cheaper to implement (2, 3). AI-DPC images of test specimens (diatoms, dried nail polish and water droplets immersed in oil) and real specimens (buccal epithelial cells and human colon cancer cell line) demonstrate that AI-DPC accentuates or retains all these advantages while being fast enough for live cell imaging. The Hilbert transform based qualitative 3D phase reconstruction method reported for DIC works well with AI-DPC images (4). The techniques are being developed further to achieve quantitative phase measurement.

Fig.1 HCT116 cells (grown on **plastic** Lab-Tek^{TM} chambered slides and mounted in medium of R.I. 1.52) were illuminated with 1.4 NA condenser and imaged with 100x 1.45 NA oil immersion objective.



Brightfield | DIC (45° shear) | AI-DPC with aperture stops placed at 45°

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