

Quantitative phase retrieval in the partially coherent Differential Interference Contrast (DIC) microscope

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The key parameters (apart from the objective's numerical aperture and the illumination wavelength) that govern imaging properties of a DIC microscope are: shear, bias, and coherence of illumination. Although coherence of illumination is of key importance for image formation in the DIC microscope, it had not been accounted properly until recently. We have proposed (1, 2) accurate spatial frequency domain models that account for partially coherent illumination used in different DIC configurations. It was shown (1) that these configurations (viz., Nomarski-DIC, Köhler-DIC used in X-ray microscopes, and PlasDIC developed by Zeiss) essentially differ, so far as their imaging properties are concerned, in their condenser geometries and hence the coherence properties of illumination. In current presentation, we demonstrate how to estimate imaging properties of experimental DIC configurations by measuring or *calibrating* shear, bias, and condenser geometry by imaging of the back focal plane (BFP) of the objective. With these measured parameters, we compute transfer functions for the DIC microscope having different settings; and demonstrate the correspondence between predicted and experimental imaging properties. We also elaborate upon the maximum allowable amount of spatial shear for being able to retrieve quantitative phase information from DIC images.

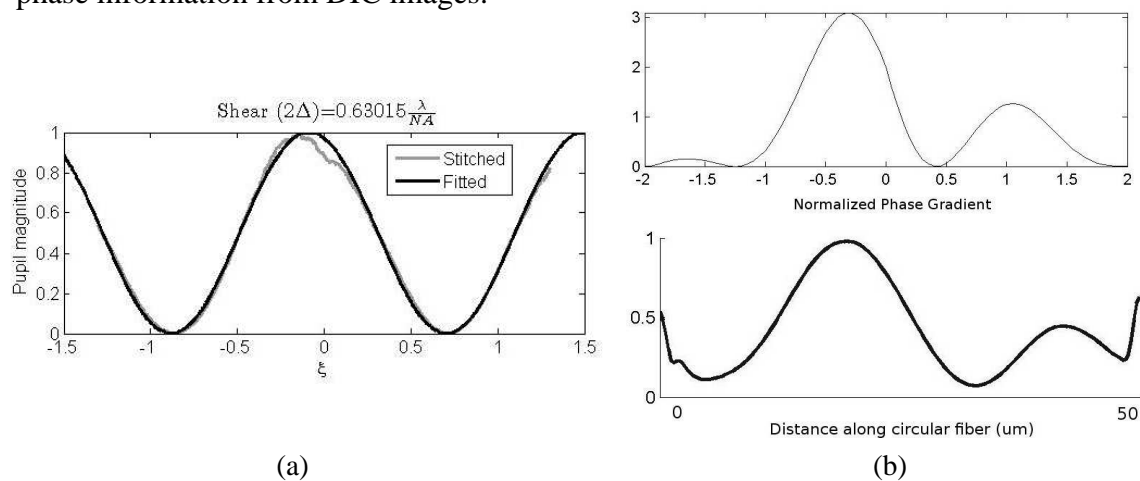


Fig. 1 (a) Measurement of the normalized value of the shear of an Olympus 20X 0.75NA objective by fitting the fringe pattern observed in the objective BFP to the model (1, Eq. 3). The fringe pattern was constructed by stitching together images of BFP at different bias values. (b) For the same objective, computed intensity profile (top) and measured intensity profile (bottom) across the width of a circular optical fiber of 50 μm diameter aligned (length-wise) perpendicular to the direction of shear. The illumination wavelength was 550 nm, illumination NA was 0.75, and bias was set to $\pi/2$.

References:

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