DIGITAL HOLOGRAPHIC MICROSCOPY FOR COMPLEX PUPIL FUNCTION EVALUATION OF HIGH NA MICROSCOPE OBJECTIVES

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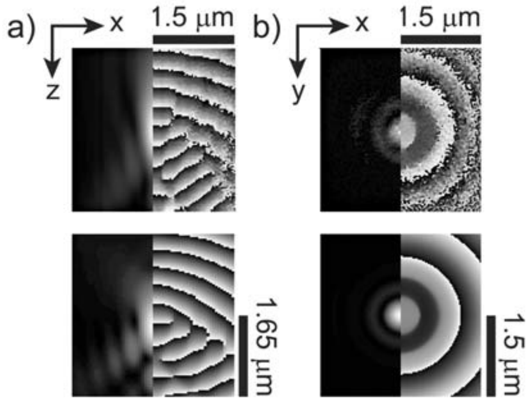

Figure 1: Axial (a) and radial (b) image comparisons in amplitude and phase: direct experimental APSF measurement (up); calculation based on pupil measurements (down).

Commonly, only the intensity point spread function is measured experimentally to describe an optical system, specifically a microscope objective (MO), neglecting the phase point spread function and loosing therefore crucial information for aberrations quantification in the system. We present here a simple experimental method, which provides an accurate and reliable 3D evaluation of the complex amplitude point spread function (APSF) of a MO, based on a single holographic acquisition [1] of its pupil wavefront. The aberration function, extracted from the holographic pupil measurements, is developed in term of Zernike polynomials and inserted in a scalar model of diffraction [2] to calculate the 3D distribution of the complex wavefront (amplitude and phase) around the focal point of the MO. Török already proposed such an evaluation, based on pupil measurement with a Twyman-Green interferometer [3] but, for the first time to our knowledge, the accuracy of the pupil-based evaluation of the 3D APSF is directly compared (Fig. 1) with a quantitative measurement of the complex APSF function performed with a second original holographic system [4]. The validity of both techniques being definitely sealed trough a comparison with a theoretical model [6]. Measurements on a 100x 1.3 NA MO are presented. The main advantage of the pupil evaluation regarding to the direct measurement techniques [4,6] resides in the single hologram required, having therefore no stability needs and a drastically reduced acquisition time.

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