PSF ENGINEERING USING A FABRICATED SQUBIC PHASE MASK TO REDUCE THE EFFECT OF SPHERICAL ABERRATION IN 3D WIDE FIELD FLUORESCENCE IMAGING

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The squared cubic (SQUBIC) phase mask (PM) was designed to engineer a point-spread function (PSF) that is insensitive to depth-induced spherical aberration (SA) [1,2]. The SQUBIC PM's design parameter *A* (i.e., the phase strength parameter) has been shown to control the stability of the SQUBIC-PSF with respect to depth-induced SA [2,3]. Theory predicts that a SQUBIC PM with a larger *A* value can render the PSF stable over a wider range of imaging depths. In earlier studies, we demonstrated the experimental implementation of a SQUBIC PM using a liquid crystal spatial light modulator (SLM) [4]. Due to the limitation of the finite pixel size of the SLM, the implementation was limited to SQUBIC PM with a lower *A* ($\leq$ 50). In this study, we investigate a SQUBIC PM (*A* = 50) fabricated on a Zeonex E48R™ polymer plate for a 63×1.4NA oil lens and emission wavelength of 515 nm. The fabricated PM was used to implement wavefront encoded (WFE) imaging in a commercial upright wide field microscope (Zeiss Axio Imager.Z1) to reduce the impact of depth-induced SA [5]. Comparison of images of 6-µm in diameter microspheres (green ring label) located at different depths below the coverslip, demonstrate the stability of the WFE system using the fabricated PM (Fig. 1). The experimental images, restored using deconvolution and a single PSF computed at 0 µm depth, show a 19% variability over 65 µm depth quantified by the correlation coefficient shown at the bottom of each restored image (Fig. 1d), while when the conventional system is used, a 43% variability is observed (Fig. 1a). In the case of the SLM implementation, for *A* = 20 and 50 (Fig. 1b&c) the WFE system shows less variability than the conventional system but only up to 30 µm depth while for higher depths artifacts are observed. Normalized intensity profiles (Fig. 1e) through the center of images from the bead at 65 µm depth, show that the SQUBIC system based on the fabricated PM provides the closest match to a 6-µm in diameter fluorescent ring (simulated true object).

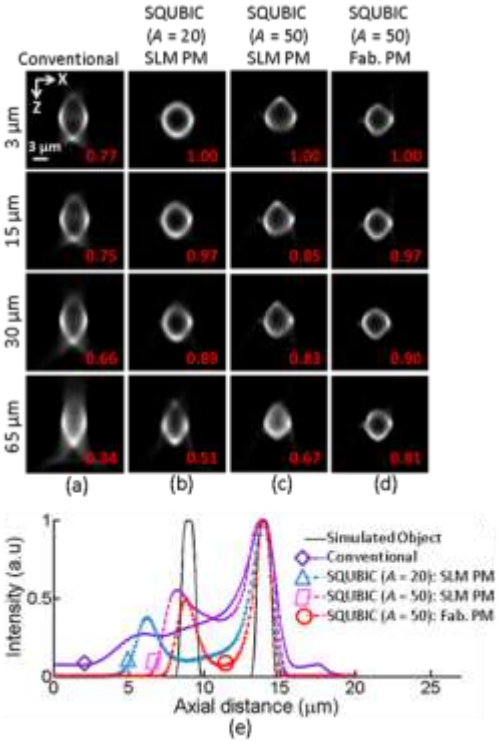

Figure 1: Experimental verification of SQUBIC-WFE system through restoration [5].

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