REDUCING THE IMPACT OF SPHERICAL ABERRATION ON EXTENDED DEPTH-OF-FIELD MICROSCOPY USING PSF ENGINEERING

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A point-spread function (PSF) engineered using wave front encoding (WFE) and a generalized cubic phase mask (GCPM) design was evaluated in this study. The GCPM design used in this study was selected to reduce the impact of depth-induced spherical aberration (SA) on extended depth-of-field (EDOF) microscopy with high NA lenses [1].

Materials and Methods: In this study, three-dimensional (3D) DV WFE-PSFs were calculated by applying the GCPM to the phase of the 2D Fourier transform of conventional complex-amplitude PSFs with increasing amounts of SA. Mean-square-error based metrics [1] were used to quantify and compare the SA sensitivity of the GCPM-PSF and the cubic-phase mask (CPM) PSF traditionally used for EDOF microscopy. Simulated images from a WFE-EDOF system using the GCPM and CPM were computed using the strata-based approach [2] and a test object with 3 small spheres (1 μm in diameter) centered at different depths (Fig. 1).

Results: The selected GCPM-based PSF for EDOF was shown to result in observations that showed a minimized impact by SA laterally and axially when compared to images simulated with the conventional or clear circular aperture (CCA) PSF. Qualitative and quantitative comparison of the EDOF images with the projection along Z of the true object show that WFE-PSF engineering with the selected GCPM design provides better performance in reducing the impact of SA than that achieved with WFE-PSFs from traditional CPM designs (Fig. 1). Also, the GCPM-based EDOF images do not suffer from the known lateral shift of object features located away from the plane of focus encountered in the traditional CPM-based EDOF images.

![Figure 1: Comparison of the true object’s projection along Z image with reconstructed EDOF (CPM and GCPM) and conventional widefield microscopy (CCA) images in the presence of SA.](image)

References

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