REDDUCING THE IMPACT OF SPHERICAL ABERRATION ON 3D MICROSCOPY IMAGING USING PROGRAMMABLE PSF ENGINEERING

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Selected point-spread function (PSF) designs, engineered using wave front coding (WFC) and a generalized cubic phase mask (GCPM), are evaluated for their effectiveness in experimental application of high NA three-dimensional (3D) microscopy in the presence of spherical aberration (SA). PSFs chosen, for extended-depth of field (EDF) microscopy and WFC computational optical sectioning microscopy (WFC-COSM), have exhibited reduced impact of depth-induced SA in simulation [1].

Materials and Methods: GCPM designs were applied using a liquid crystal spatial light modulator (SLM) at the pupil plane of a Zeiss AxioImager microscope. Mean-square-error based metrics [1] were used to quantify and compare the SA sensitivity of computed and experimentally determined 3D depth-variant (DV) GCPM-PSFs. Test preparations of fluorescent microspheres (using unresolved and resolved microspheres) at different controlled depths were used to acquire experimental PSFs from the WFC-EDF and the WFC-COSM systems with and without application of the GCPM.

Results: SLM driven implementation of the DV PSFs allows programmable selection of PSFs during experimental image acquisition. Using this approach to apply WFC-PSF engineering with the selected GCPM designs produces an effect which is consistent with previously simulated results. These have shown minimized SA laterally and axially when compared to images acquired with the conventional PSF.

Figure 1: Adaptation of the Zeiss AxioImager microscope for experimental realization of GCPM designs: a) front view of microscope and optical relay via the optional side port; b) top view of optical relay with the programmable SLM located at the Fourier plane of a one-to-one magnification 4-f system imaging system; and c) example GCPM optimized for COSM.

References

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