EXTRACTION OF DEPTH INFORMATION IN THE PRESENCE OF SPHERICAL ABERRATION USING DOUBLE-HELIX POINT SPREAD FUNCTION CODING IN 3D FLUORESCENCE MICROSCOPY IMAGING

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Point spread function encoding with a double helix (DH) phase mask in the imaging path of a microscope is a joint computational-optical approach to extract depth information from fluorescence images. In this approach, the depth at which a point source of light is located is encoded in a rotating pattern in the point-spread function (PSF). Depth information can be obtained by computing the angle of rotation and mapping it to an accurate depth distance through a calibration curve [1] or by use of pattern matching techniques [2]. Systems modified with a DH mask are characterized by a double-helix PSF (DH-PSF) and have been successfully used in different applications [1-4]. In this study, we investigate the effect of increasing spherical aberration (SA) on: 1) the DH-PSF, 2) the calibration curve computed from DH-PSF, and 3) on the ability to extract depth information from images that include SA.

A computer-generated phantom comprising of beads, 100 nm in diameter located at different depths in a water medium, was used to simulate images based on a depth variant forward model implemented in the COSMOS software [5]. The refractive index and depth location of the beads contribute to the amount of SA in the images of the phantom. Conventional PSFs with SA were computed for a 63X/1.4 N.A oil-immersion lens for a wavelength of 633 nm. DH-PSFs were computed by applying a DH phase mask to the generalized pupil function extracted from the conventional PSF. Calibration curves were generated from the DH-PSFs by computing the rotation of the pattern at each focus depth. Our results show that a single section image can be used to obtain information regarding the location of several beads in the object volume even in the presence of SA. The depth information is preserved and accurately retrieved with an appropriate adjustment to the calibration curve. Thus, a microscope modified to include a DH mask provides precise information about the 3D location of beads located within an extended object, which can be extracted using only a single 2D image even in the presence of SA.

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[5] COSMOS Software Package, URL: http://cirl.memphis.edu/cosmos.

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