3D DEPTH VARIANT PSF ANALYSIS AND INTERPOLATION USING ZERNIKE MOMENTS

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KEY WORDS: Point Spread Function, depth variant PSF, 3D deconvolution, Zernike moments.

1. Abstract:

The images acquired using fluorescent microscopy are subject to a blurring effect, as the acquired image is the result of a convolution process between the object and the optical system point spread function commonly named PSF. Many deconvolution algorithms have been developed to reverse the blurring effect, for these classical algorithms, the PSF is assumed to be space invariant [1].

In 3D microscopy the PSF changes with depth [2], neglecting these variations in deconvolution, using thick specimens, introduces large errors in the restored images. The PSF non-invariance has been taken into consideration in some deconvolution algorithms [3] [4], in which, the knowledge of the PSF variation and measuring it at various depths is essential for an accurate restoration.

Measuring PSFs at various depths is still a challenging task. A method is proposed based on the general theory of moments using Zernike polynomials as a decomposition basis in order to describe the acquired PSF [5] and to study there's variations over depth. Furthermore unknown intermediate PSF can be interpolated using only a limited number of known ones. The presented method has been tested on simulated and an acquired set of PSF at different depth. Measured PSF are acquired using 100nm beads embedded in a photo-polymer sample having a 30µm thickness, prepared in collaboration with chemist from IS2M laboratory. These tests demonstrate the possibility to obtain an accurate PSF estimation at different positions using only few known PSF. This work aims to facilitate the use of depth-variant deconvolution methods. Furthermore it can be used to estimate PSF variations induced with other optical parameters, e.g the changes in the immersion oil refractive index due to ambient temperature variations.

2. REFERENCES:

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