STATICAL COMPENSATION OF SAMPLE-INDUCED SPHERICAL ABERRATION IN SCANNING MICROSCOPY BY WAVEFRONT CODING

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Modern live cell fluorescent techniques have opened new possibilities in the analysis of thick specimens. Although a single two-dimensional (2D) image may be sufficient for many studies, the reduced depth of field (DOF) of classical microscopes is a major limitation for a single-shot inspection of the whole specimen. The extension of this capability has been an active research area in recent years [1]. In particular, the so-called wavefront coding (WFC) consists of a combination of an apodization of the pupil of the system, to decrease the sensitivity of the impulse response of the optical system to the defocus, plus a deconvolution procedure to achieve the final 2D image of the thick sample. This technique increases significantly the maximum thickness that can be registered sharply in a single-shot image. Note that the deconvolution algorithms used in this field rely on the invariance of the three-dimensional impulse response of the imaging system at different depths inside the sample. However, the effect of aberrations generated by the index mismatch between the specimen and the surrounding media invalidates this hypothesis, even in high optical sectioning systems as scanning confocal or multiphoton microscopy. This fact limits the effective DOF achieved with these techniques. Although *sequential* compensation of these aberrations can be performed for each particular plane inside the sample, it is not possible to achieve a global correction for the whole sample at the same time.

Considering the primary spherical aberration as the major effect of the above index mismatch, we present in this work an apodization technique that provides the optical system with a quasi-invariant impulse response. The pupil filter that we propose is a mapped version of a radial cubic-phase mask [2]. The result is an impulse response not only with low-sensitivity to defocus, as in the conventional WFC, but also almost-independent of the position of the sample point inside the thick specimen. After the application of the same deconvolution techniques as in classical WFC, our hybrid optical-digital proposal leads to an actual extension of the DOF of the microscope.

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