MEASUREMENT AND SIMPLE ADJUSTMENT OF SPHERICAL ABERRATION FOR CONFOCAL MICROSCOPY

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Abstract

Aberrations of the wavefront are known to compromise the resolution and signal intensity in confocal and multiphoton microscopy. When aberrations are present the point spread function is distorted and the performance of the instrument is compromised.

One has to distinguish between static and specimen induced aberration. The static component may be caused by bad alignment or inappropriate design of the optical system while the specimen induced fraction is induced by the sample itself. Aberrations can be measured and monitored using interferometry [1] or modal wavefront sensors [2]. However, the required hardware is not easily integrated into a standard microscope or expensive.

Among the different types of aberration the spherical component of the aberration is of special importance since the axial resolution is especially sensitive to it [3]. Spherical aberration depends on the focussing depth and may be caused by a mismatch in refractive index between the specification of the lens and the immersion fluid (for example when an oil immersion lens is used to image a water-based sample) or if the coverslip does not have the appropriate thickness.

Some lenses are equipped with a correction ring that allows for the adjustment of the coverslip thickness and hence the spherical aberration. However, the choice of the optimum setting can be difficult for the user: a change in the setting for the spherical term causes an axial shift of the imaging plane, the setting is sensitive and the commonly used image intensity is only a crude and sometimes inappropriate measure for the adjustment quality.

We have measured the spherical aberration component in conjunction with the shift in focal position for a typical lens with a correction ring over the full correction range. A phase stepping interferometer was built to record the wavefront data in the pupil plane of the lens and the Zernike mode components including the spherical term were extracted from the wavefront. The data for the spherical aberration is compared to results from theory and simulations for the aberrated pupil functions were calculated.

We suggest a new method for the optimisation of the adjustment of the spherical aberration that is based on evaluation of the reflection on the top surface of the microscope slide. We have implemented an automated software algorithm that allows the user to tune the spherical aberration setting of the confocal system without much effort. It simplifies the optimisation process and also improves its accuracy.

[1] M. Schwertner, M.J. Booth, M.A.A. Neil and T.Wilson: Measurement of specimen-induced aberrations of biological samples using phase stepping interferometry. Journal of Microscopy, 213(1):11-19, 2004.

[2] M. J. Booth, M.A.A. Neil, Rimas Juškaitis and Tony Wilson: Adaptive aberration correction in a confocal microscope. PNAS 99:5788-5792, 2002

[3] T. Wilson and A.R. Carlini: The effect of aberrations on the axial response of confocal imaging systems. Journal of Microscopy, 154(3):243-256, 1989.