

Aberration effects in quantitative confocal microscopy and simulation of aberration correction devices

Michael Schwertner, Martin Booth, Tony Wilson
Department of Engineering Science University of Oxford, OX13PJ
Email: Michael.Schwertner@eng.ox.ac.uk

Keywords: Confocal microscopy, specimen induced aberrations, geometric distortions, fluorescence signal intensity

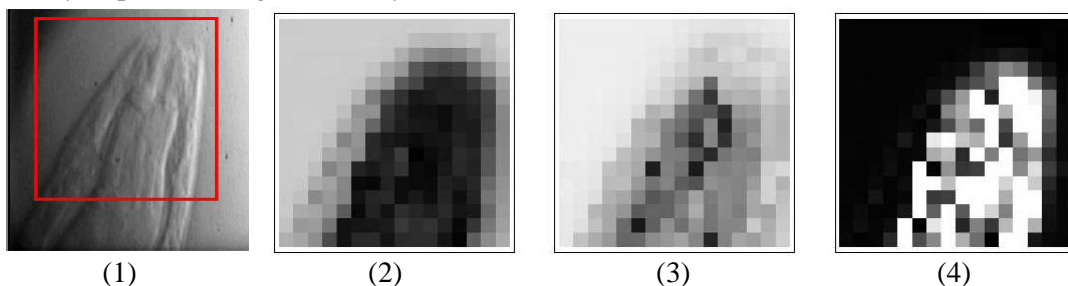
Aberrations have a strong influence on imaging quality in confocal and multiphoton microscopy. Among the different sources of aberrations, the specimen itself gives the largest contribution in most practical settings. Aberrations can arise from variations in refractive index, or a mismatch in refractive index, for example between the immersion medium and the embedding medium. The effects are particularly strong for high NA lenses, which are typically used in confocal and multiphoton microscopy to obtain high-resolution images. Aberration effects include a reduction of signal level, compromised resolution and also geometrical distortions of the image.

The technique of adaptive optics (AO) can correct for aberrations and such arrangements contain a wavefront sensing and correction devices. In order to design such systems one needs to know about the nature of the aberrations that occur for typical biological specimens.

Therefore we built a phase stepping interferometer to measure the wavefront in the pupil plane of the lens under high NA conditions[1]. A convenient way to describe aberrations is the decomposition into a combination of Zernike polynomials. These are a set of orthonormal mathematical functions, where some of the polynomials correspond to classical terms such as astigmatism, coma or spherical aberration. The results show that most aberrations can be described by a relatively low number of lower order Zernike polynomials.

Interferometric data was taken from typical biological specimens and the Zernike mode content was analysed. The three lowest order modes, tip, tilt and defocus, do not affect resolution or signal intensity but they cause a lateral (tip, tilt) or axial (defocus) displacement of the focal spot, resulting in a geometrical distortions of the confocal image. We quantified these aberration effects that can influence the accuracy of spatial measurements. Deviations in the order of a few microns were found.

Furthermore, we calculated the loss in signal level due to aberrations. The drop in signal has important implications for quantitative fluorescence intensity measurements and cannot be ignored. We simulated the benefit of AO correction for different correction devices, for example Zernike mode based correction using a deformable membrane mirror and segmented / pixellated correction devices, e.g. a segmented mirror or a SLM. Results show that for several of the specimens investigated AO correction may improve the signal level by a factor of about 10, in a few cases even more.



The Figure shows an example for a *C. elegans* specimen where wavefronts were recorded on a 16x16 grid. (1) is the transmitted light image, the box represents the scanned area of 50x50 micron. (2) displays the focal intensity before and (3) the recovered intensity after a simulated AO correction. Panel (4) shows the estimated signal improvement factor for a fluorescent confocal microscope. The grey scale ranges are: [0,1] for (2) and (3); [0..20] for (4).

Reference

[1] M. Schwertner, M. J. Booth, and T. Wilson: Characterizing specimen induced aberrations for high NA adaptive optical microscopy, *Optics Express*, Vol. 12, No. 26, Dec 2004, Page: 6540 - 6552