Adaptive optics in high numerical aperture microscopy

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Introduction

The performance of optical microscopes is degraded by refractive aberrations arising from the imperfections in the optical system, differences in bulk refractive index and the inhomogeneous refractive index structure of the specimen itself [1]. In optical sectioning microscopes, such as confocal and multiphoton systems, and also in other optical devices employing high numerical aperture (NA) lenses, these aberrations can be large and the use of high NA lenses can also result in a dependence of the phase and intensity on the polarization state of the light [2]. A number of different schemes employing adaptive optic components to correct such phase aberrations have been proposed, some of which rely on optimization of the acquired image [3] and others which use direct wavefront sensing [4-6].

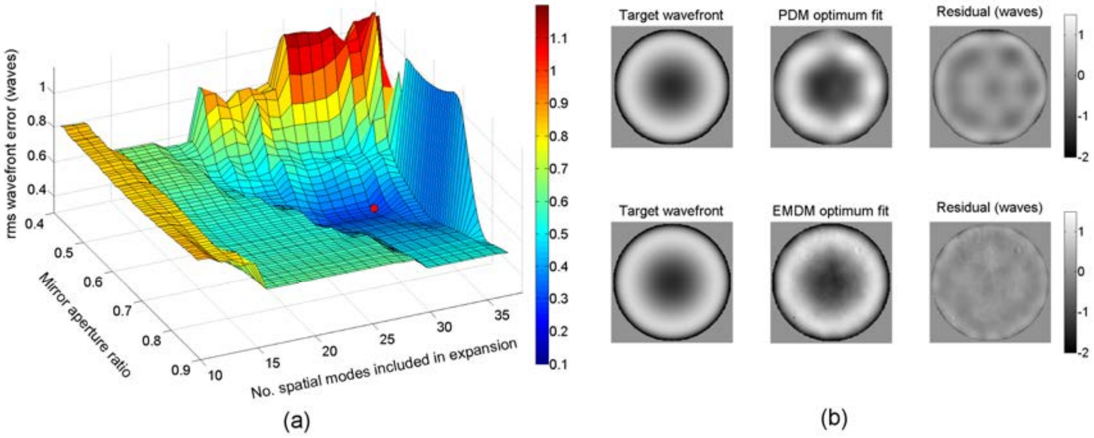
We investigate the measurement and correction of wavefront aberrations in systems employing high (NA) lenses, particularly biological microscopes. We quantify the capability of commercially available deformable mirrors for correction of the spherical aberration introduced by a planar refractive index mismatch between the objective immersion medium and the specimen itself. We also describe an experimental system for wavefront sensing through a microscope objective in order to explore the measurement of phase aberrations in such systems.

Characterisation of deformable mirrors

A Fizeau interferometer was used to measure the influence matrices of two commercially available deformable mirrors (DMs): a 52 actuator electromagnetic mirror with a 15 mm clear aperture (Imagine Eyes) and a 37 actuator piezoelectric mirror with a 50 mm clear aperture (Flexible Optical B.V.). Assuming a linear model of mirror behaviour we computed the optimum correction achievable for several different microscope objectives focussing through planar refractive index boundaries (see fig.1) [7].

Wavefront Sensing

We have constructed an upright scanning epi-microscope system for measuring wavefront aberrations through a high numerical aperture microscope objective. The objective lens focuses light from a 635 nm fibre coupled laser diode into the specimen. Light emitted from the specimen is collected by the same objective and sent, via a beamsplitter and a 4f optical relay, to an imaging camera and a Shack-Hartmann wavefront sensor which record the intensity and the wavefront in the pupil plane of the objective lens. High precision translation stages allow the specimen to be moved in three dimensions relative to the focused spot. We have used this setup up to measure wavefronts scattered from objects such as fluorescent microspheres and fluorescent solutions.



(a) (b)

Fig. 1 (a) Residual wavefront error (WFE) vs. mirror aperture ratio and number of spatial modes in the mirror control matrix for piezoelectric DM correcting spherical aberration from an NA = 1.2 oil immersion objective imaging 100 µm into water. (b) Target phase (left), best mirror fit (centre) and residual WFE for piezoelectric (top) and electromagnetic (bottom) DMs

Conclusions

We have investigated wavefront sensing and wavefront correction in high numerical aperture microscopy. The ability of two commercially available DMs for correcting depth aberrations arising from focussing light through a planar refractive index mismatch has been quantified by interferometric measurement of the DM influence matrices and projection of the orthogonal modes of the mirror onto simulated phase aberrations. Both DMs substantially increase the focussing depth at which diffraction limited imaging is possible; an optimum correction is achieved when a ring of actuators are situated just outside the illuminated mirror pupil. A system for measuring the wavefront aberration through a microscope objective has also been described.

References

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