

SPECIMEN INDUCED ABERRATIONS: NEW METHODS FOR THEIR MEASUREMENT AND REPRESENTATION

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Specimen induced aberrations are known to affect the imaging performance of confocal and multiphoton microscopes by reducing signal level, introducing distortion and reducing resolution. These aberrations are distortions in optical wave fronts that are introduced when the light is focussed through a change in refractive index, for example between the cover glass and the mounting medium, or when the light propagates through regions of the specimen with varying refractive index. These effects can be compensated through the use of adaptive optics whereby one measures and removes aberrations [1]. The design and optimisation of an adaptive optics system requires knowledge of the nature of aberrations that are introduced by typical specimens. For this reason, we have previously implemented a low numerical aperture (NA) phase-stepping interferometer system to measure the distortions induced in wave fronts as they propagate through various specimens [2]. The measured aberrations are conveniently represented as a combination of Zernike polynomials, a set of orthonormal mathematical functions that correspond to traditional aberrations such as coma, astigmatism and spherical aberration.

We describe here the development of the interferometric measurements to use high NA objective lenses. Most modern confocal microscopes used in the life sciences employ high NA oil or water immersion lenses in order to obtain the highest resolution images. It is also known that high NA systems are more susceptible to the effects of aberrations. The results show that the specimen-induced aberrations are mostly dominated by the low-order Zernike modes. In general, the most prominent are the three modes tip, tilt and defocus. These correspond to the lateral (tip, tilt) and axial (defocus) displacement of the focal spot. In confocal and multiphoton microscopes, the presence of these modes leads to distortion of the image. The higher aberrations cause degradation of the point spread function, leading to reduced signal level and resolution.

It is also apparent for certain specimens that there are correlations between different aberration modes. It is well known, for example, that focussing through a planar refractive index mismatch introduces a certain combination of spherical aberration modes that depends upon the refractive indices of the two media and the focussing NA [3,4]. Through a combination of empirical results and theoretical modelling of structures we show that correlations between other modes should also be seen. Analysis of the wave front data allows us to ascertain whether the Zernike polynomials really are the optimum set of functions for describing aberrations in biological specimens. Furthermore, through application of a singular value decomposition algorithm we can derive new orthogonal sets of aberration modes that will lead to more robust adaptive optics systems for microscopy.

References

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