IMAGE BASED ADAPTIVE OPTICS FOR MICROSCOPY

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Microscopic imaging of thick biological specimens is often detrimentally affected by specimen-induced aberrations. In the simplest case, these aberrations arise from a refractive index mismatch between the immersion and mounting media. In other situations, the aberrations arise from variations in refractive index within the specimen. These aberrations cause loss of signal and reduced resolution. Aberrations can be corrected using adaptive optics, where a deformable mirror introduces equal but opposite aberrations into the optical path. Aberration correction can be performed by reconfiguring the deformable mirror and using the fluorescence signal as feedback, effectively maximising the fluorescence intensity. Such adaptive optics systems, which do not employ a traditional wave front sensor, show promise for use in microscopy. However, the degree to which aberrations affect the intensity is related to the distribution of fluorescence in the specimen. For example, signals from point-like objects are affected more by aberrations than equivalent signals from planar or volume objects. As a consequence, it is not straightforward to determine the specimen induced aberrations without knowledge of the specimen structure. We investigate these effects and discuss the implications for adaptive optical microscopy of biological specimens.

Since a universal method of aberration determination must be independent of object structure, we introduce a different approach. It is often beneficial to investigate the imaging process in the Fourier domain, by considering the spatial frequency content of the object and the optical transfer function of the imaging system (Figure 1). Although aberrations are most often expressed as a series of Zernike polynomials, we find that that an expansion in terms of polynomials derived by Lukosz is more appropriate for representation of the imaging of low spatial frequencies. By using this Lukosz expansion to optimise the low frequencies, we derive robust schemes for aberration measurement and correction that do not depend on the specimen structure.

Figure 1: Incoherent transmission microscope images (a and c) and Fourier transform modulus (b and d, logarithmic grey scale) of a scattering specimen without aberrations (a and b) and in the presence of a random aberration (c and d). The circle shows the spatial frequency cut-off of the imaging system.