ADAPTIVE OPTICS FOR NONLINEAR MICROSCOPY OF BIOLOGICAL SPECIMENS AND PHOTONIC STRUCTURES

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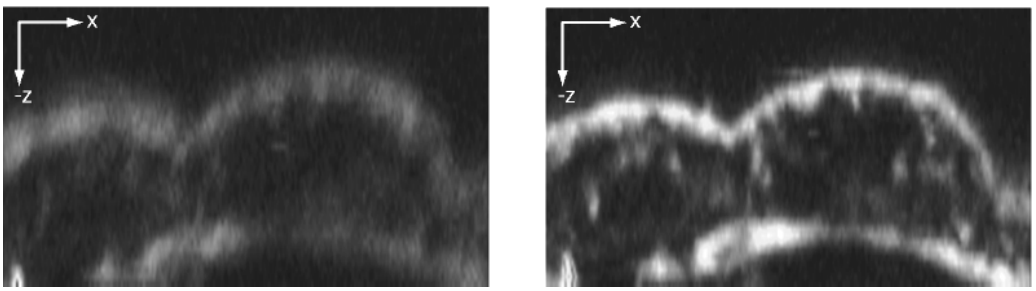
Specimen-induced aberrations are frequently encountered in high resolution microscopy, particularly when high numerical aperture lenses are used to image deep into specimens. These aberrations distort the focal spot causing a reduction in resolution and, often more importantly, reduced signal level and contrast. This is particularly problematic in multiphoton microscopies, such as two-photon fluorescence or third harmonic generation (THG), where the non-linear nature of the signal generation process means that the signal level is strongly affected by changes in the focal intensity. The techniques of adaptive optics have been used to measure and correct the aberrations, restoring image quality in a number of microscopes [1]. We present here in results from an adaptive harmonic generation microscope with applications in different areas. As all of these applications require focusing at depth within an inhomogeneous specimen, they suffer from the effects of aberrations. Adaptive optics can be used to compensate the aberrations, leading to improved signal levels and resolution.

THG microscopes are capable of providing high-resolution, three-dimensional, label-free images of biological specimens [2]. The THG signals are generated through the non-linear optical properties of the specimen, so images are obtained without the need for exogenous markers. This method is therefore particularly promising in areas such as developmental biology, where living specimens must be observed over long periods with minimal perturbation [3]. We have used the adaptive harmonic microscope to image developing mouse embryos over long periods (Fig. 1) [4]. Harmonic generation is also produced by structures in photonic devices, between regions of differing optical properties. We have used this to reveal the structure of waveguides in bulk substrates and the structural properties of liquid crystals.



(a) (b)

Figure 1: xz plane THG images of mouse embryos from a depth of around 90 μm: (a) before and (b) after correction of specimen induced aberrations (image width 40μm).

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