ADAPTIVE OPTICS FOR ABERRATION CORRECTION IN HARMONIC GENERATION MICROSCOPY

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Harmonic generation microscopes are capable of providing high-resolution, three-dimensional, label-free images of biological specimens. Third harmonic generation (THG) images demarcate interfaces and spatial variations in optical properties. Second harmonic generation (SHG) signals are produced by a number of biological structures, such as microtubules, myosin or collagen. The harmonic 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.

Specimen-induced aberrations are frequently encountered in high-resolution microscopy, particularly when high numerical aperture lenses are used to image deep into biological 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 harmonic generation microscopy, where the non-linear nature of the signal generation process means that the signal level is strongly affected by changes in the focal spot intensity. Furthermore, for combined SHG and THG imaging it is desirable to use infrared excitation wavelengths around 1200nm, producing SHG and THG emission at 600nm and 400nm, respectively. As this is typically outside the specification of most objective lenses, system aberrations can also be significant.

We have applied the techniques of adaptive optics to measure and correct aberrations in harmonic generation microscopes, restoring signal level and image quality. As aberration correction leads to more efficient signal generation, the illumination laser power can be reduced. The energy absorbed by the sample is therefore lower leading to reduced phototoxic effects when imaging living specimens. This in turn could permit an increase in the period over which specimens could be observed. To this end, we have developed efficient adaptive optics schemes for harmonic generation microscopes that help minimize the specimen exposure.