Adaptive Optics for Microscopy and Photonic Engineering

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Introduction

We describe advances in adaptive optics for use in microscopy and laser-based micro/nano-fabrication of photonic devices. We have developed wavefront sensorless adaptive optics systems for multiphoton microscopes for application in biological research. These microscopes have been used in the imaging of thick tissue specimens, ensuring that image quality if maintained over a range of focusing depths.

We have also implemented adaptive systems for compensation of aberrations encountered when laser fabricating three-dimensional structures, such as photonic crystals and waveguides, inside bulk materials. Aberration correction has also been combined with adaptive parallel fabrication methods to improve fabrication speeds.

Adaptive optics for high resolution microscopy

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 harmonic generation, 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 [1,2,3]. In imaging systems like microscopes, direct wave front sensing is not straightforward and wave front sensor-less schemes are often employed. In these systems, the adaptive element is reconfigured in order to maximise a metric related to image quality. We describe the design of efficient schemes for these microscopes that minimize the specimen exposure. This process involves the derivation of aberration modes that are specific to each type of adaptive microscope.

We present here in results from sensorless adaptive multiphoton microscopes 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. Specific examples are shown for imaging of fixed mouse embryos in two-photon fluorescence microscope. Imaging of live embryos in an adaptive second and third harmonic generation microscope is also demonstrated (Figure 1).

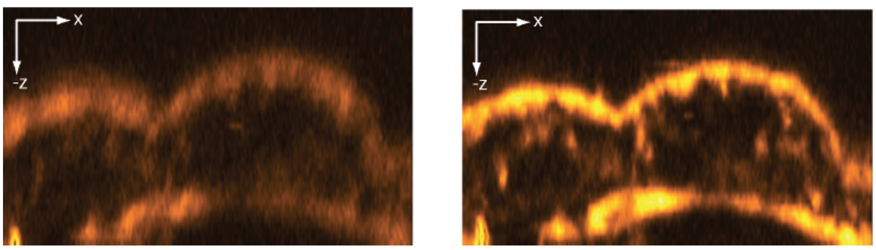

Figure 1: xz plane third harmonic generation microscope images of mouse embryos before and after correction of specimen induced aberrations (image width 40μm).

Adaptive optics for photonic fabrication

Various techniques exist for direct laser writing of micron-scale three-dimensional structures within bulk substrates [4]. These techniques usually employ a femtosecond pulsed laser and high numerical aperture (NA) focussing optics to produce focal spots smaller than one micrometre in all dimensions. Combined with non-linear and thresholded fabrication processes, feature sizes can be reduced to around 100nm. Applications of these techniques include the fabrication of photonic crystals, metamaterials, waveguide structures and microfluidics.

All of these applications require focusing of the laser beam, using a microscope objective lens, into the bulk of the fabrication medium. Any refractive index difference between the fabrication and objective immersion media causes refraction at the interface, which in turn leads to aberration of the laser focus. These aberrations can severely distort the focus, in particular through elongation along the optical axis, and cause a significant drop in peak intensity. Consequently, the efficiency and accuracy of fabrication are severely compromised. The aberrations become more severe as one focuses deeper into the material, significantly restricting the thickness of three-dimensional structures that can be fabricated by this method.

We have demonstrated adaptive aberration correction for direct laser written fabrication in a range of substrates. These include wave-guiding structures in glass and fused silica, photonic crystals using void structures in lithium niobate, and graphite structures within diamond. A aberration measurement and correction scheme using feedback from the focal plasma emission was introduced [5]. This correction enabled the fabrication of extended three-dimensional high fidelity structures over a range of depths far exceeding that achieved with conventional optical systems.

One limitation of the direct laser write method is its inherent lack of speed. Three dimensional structures are constructed in a point-by-point manner whilst scanning the laser focus through the substrate. The speed can be considerably improved by creating an array of focal spots, each of which can fabricate features in parallel. We show how holographic multi-spot methods can be combined with spatially dependent aberration correction to produce three-dimensional distributions of more than a hundred features in a single exposure [6], as demonstrated in Figure 2.

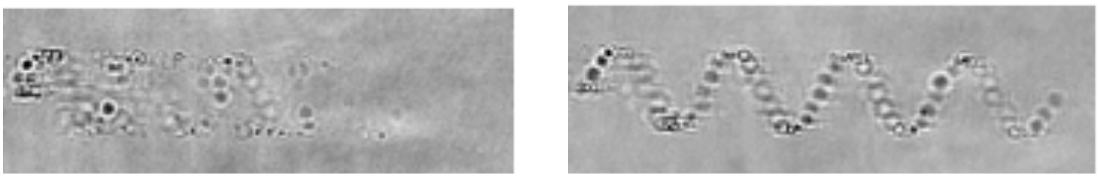

Figure 2**:** Single shot fabrication of 3D helical structures in fused silica using holographic beam shaping. The voids were fabricated without (left) and with (right) depth dependent aberration correction. The images were taken using a transmission microscope from the side aspect with the optic axis running from left to right. Image width is 100μm.

References

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