Extended depth-of-focus with induced spherical aberration improves 3D multiphoton microscopy

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Purpose

The resolution and contrast of 3D multiphoton imaging are limited by aberrations[1]. Multiple scattering and changes in the refraction index affect the penetration depth and the intensity of the illuminating femtosecond laser beam, reducing significantly the probability of nonlinear processes. Adaptive Optics (AO) has been reported as a useful strategy to increase depth penetration in both two-photon excitation fluorescence (TPEF)[2] and second harmonic generation (SHG)[3]. An ideal situation would require plane-by-plane measurement and correction of aberrations, however this implementation is complex. To overpass this limitation the aim of this work was to investigate the influence of manipulating the aberrations of the laser beam while performing multiphoton imaging. In particular the effect of the spherical aberration (SA) to extend depth-of-focus improving 3D images is discussed in detail.

Methods

The experimental system combines a research multiphoton microscope combined with an AO module: a Hartmann-Shack (HS) wavefront sensor and a deformable mirror (DM)[3]. A mode-locked Ti:Sapphire laser beam used as illumination source passes the AO module before reaching the XY-scanning unit and entering the microscope. An additional Z-scan motor attached to the microscope objective allows to record stacks of images at different depths within the sample to obtain 3D reconstructions (volume renderings). The backscattered signal (both SHG and TPEF) from the sample passes a dichroic mirror, the corresponding interference filter and arrives to the detection unit.

The aberrations of the laser beam are estimated from the recorded HS images and the DM compensates them in closed-loop by appropriately changing its shape. The system can be also used to produce a desired type or amount of aberration. In particular, the AO module is used to produce pre-defined values of SA while correcting for the rest of the aberrations present in the beam. To establish the relative impact of the induction of SA in the multiphoton microscopy images different image quality parameters were used.

Although non-biological and biological samples (providing both TPEF and SHG signal) were involved in the experiment, only results on the former are presented below. For all samples, nonlinear microscopy images were acquired for different depth positions. For the non-biological samples the 0-μm reference position was chosen as the plane with the maximum total intensity level (control plane).

Results

Aberration correction produced a significant increase in the recorded TPEF signal at every location within the sample (Fig. 1). For both experimental conditions (AO off and on) only one plane provides the highest signal level (control plane, 0-μm position). Images on the right show that more details were visible when AO was in operation independently of the depth location.

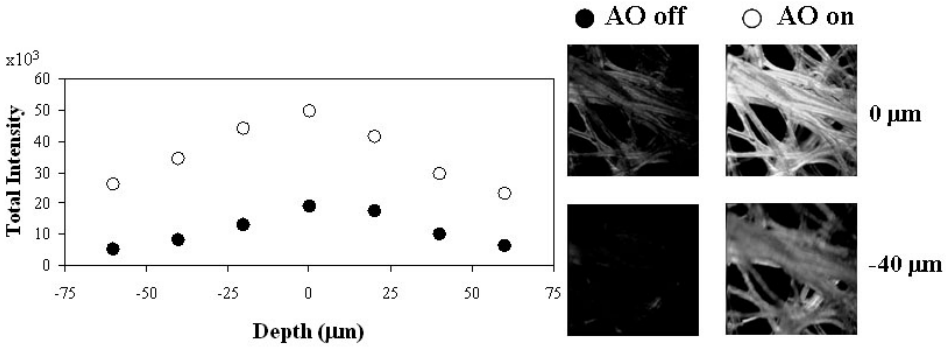

Fig. 1. Impact of AO correction across depth on TPEF microscopy imaging (stained paper).

Fig. 2 shows the impact of inducing a controlled amount of SA compared to the case of complete aberration correction. There was a reduction of 20% in TPEF signal at the control plane after inducing SA. This effect however did not take place at every depth position as observed at the locations marked with arrows. At those locations, when going further from the control plane, the behaviour was reversed and better TPEF signal was obtained.

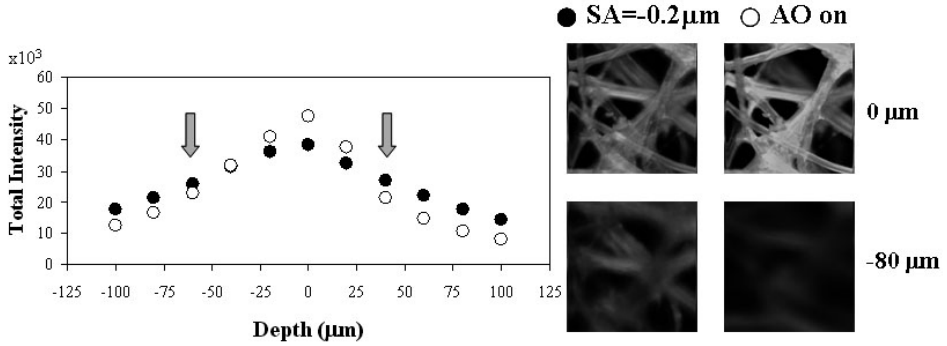

Fig. 2. Effect of inducing -0.2 μm of SA compared to the case of complete correction.

Conclusions

3D multiphoton microscopy techniques might significantly be improved with an accurate optimization of the aberration in the illuminating beam. In particular, the induction of controlled amounts of SA would extend depth-of-focus improving deeper images. At particular locations, the TPEF signal for SA-induced conditions is higher than that corresponding to a complete correction of aberrations. Successive planes within the sample provide better signal, what leads to an increase in the depth-of-focus independently of the specimen-induced aberrations, allowing a more in-depth imaging what is essential in multiphoton microscopy.

References

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