ADAPTIVE OPTICS MULTIPHOTON IMAGING COMBINING SPHERICAL ABERRATION AND SQUARED CUBIC PHASE MASKS

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Multiphoton (MP) microscopy is mainly limited by specimen-induced aberrations. Among them spherical aberration (SA) is the dominant term at deeper layers within a sample [1]. Adaptive optics has been demonstrated to improve MP imaging performance [1,2]. Wavefront coding techniques (or alternatively, pupil masks) have also been used to manipulate the wavefront and reduce the impact of SA when imaging thick tissues [3]. In particular, a symmetric squared cubic pupil phase mask (known as SQUIBIC) was able to produce a depth-invariant focal spot with noticeable enhanced confocal microscopy images [4]. Here, we propose a wavefront sensor-less approach based on combining SQUBIC masks and SA patterns to further improve the quality of MP images.

A spatial light modulator was implemented into the illumination pathway of a custom-build MP microscope to manipulate the incident laser beam wavefront [2]. This was used to generate phase maps with different amounts of SA, and to produce a number of SQUBIC masks attending to its design parameter [4]. Individual or combined SA and SQUIBIC patterns were systematically generated during image recording. A set of image quality metrics was used to evaluate the impact for different nonlinear signals and depth locations within the samples under study.

Both negative SA maps and SQUBIC patterns were able to improve MP images. However, results show that particular combinations of both yield improved images, better that those obtained when using those wavefront patterns separately (see Figure 1 as an example). Moreover, those controlled combinations of phase conditions led to an increase in depth-of-focus, noticeable better than that obtained just using SA [1], independently of the specimen-induced aberrations.
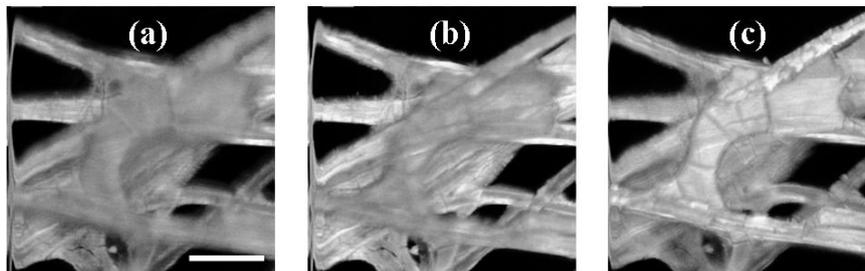

Figure 1: MP images (cellulose). (a) Original; (b) optimum SA pattern; (c) optimum combination of SA and SQUBIC. Bar length: 50 µm.

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