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Multiphoton Image Enhancement with Variable Squared Cubic Phase Masks

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Abstract: We propose the combination of squared cubic phase profiles and spherical aberration patterns to further improve the quality of multiphoton microscopy images. The benefit will be analyzed through the use of objective image quality metrics.

1. Introduction

Multiphoton (MP) microscopy provides inherent confocality and reduced photodamage, what is crucial for the analysis of thick biological samples [1]. Despite this, imaging at deeper locations is often challenging due to degradation of the focal spot imposed by the specimen-induced aberrations [2], in particular spherical aberration (SA) [3]. Although these limitations are usually overcome by using adaptive optics [2,3], other alternative methods to improve microscopy imaging performance include the use of wavefront encoded techniques [4]. Although symmetric squared cubic pupil phase masks (known as SQUBIC) produce a depth-invariant focal spot with noticeable enhanced microscopy images [5,6], to the best of our knowledge they have never been used in a MP microscope. Here, we propose to implement a wavefront sensor-less approach using variable SQUBIC masks to further improve MP imaging effectiveness.

2. Methods

A liquid-crystal-on-silicon spatial light modulator (LCoS-SLM) was implemented into the illumination pathway of a custom-build MP microscope to manipulate the incident laser beam wavefront [7]. A schematic diagram is shown in Fig. 1. This uses a Ti:sapphire femtosecond laser (800 nm, 76 MHz) as illumination source. The beam was spatially modulated by the LCoS-SLM, passed the XY scanning mirrors (SM1 and SM1) and the microscope objective. A Z-motor was attached to the objective to either collect stacks of XY images at successive depths or allow the system operating in a tomography imaging mode. A set of spectral filters placed in front of a photomultiplier tube (PMT, detection unit) was used to isolate the different MP signals. The instrument was controlled and synchronized through a custom LabView™ interface. Image processing was performed with MatLab™.

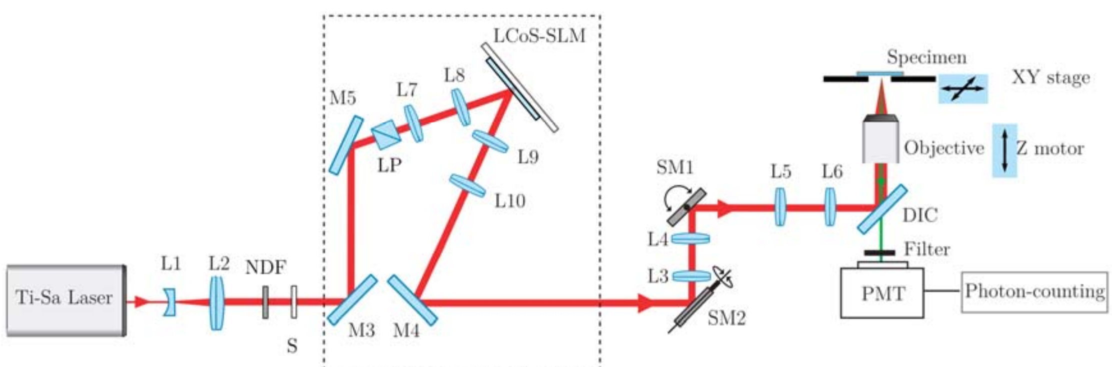

Fig. 1: Schematic of the MP microscope used for the purpose of this work. Liquid-Crystal-on-Silicon Spatial Light Modulator, LCoS-SLM. L1-L10, lenses; NFD, neutral density filter; SM1-SM2, scanning mirrors; M3-M5, mirrors; LP, linear polarizer; DIC, dichroic mirror; PMT, photomultiplier tube.

The SLM was used to produce a series of SQUBIC patterns attending to its design parameter (A) [5]. These SQUIBIC masks were projected both individually and in combination with different amounts of SA (Fig. 2). Different image quality metrics were used to evaluate their impact on MP imaging effectiveness.

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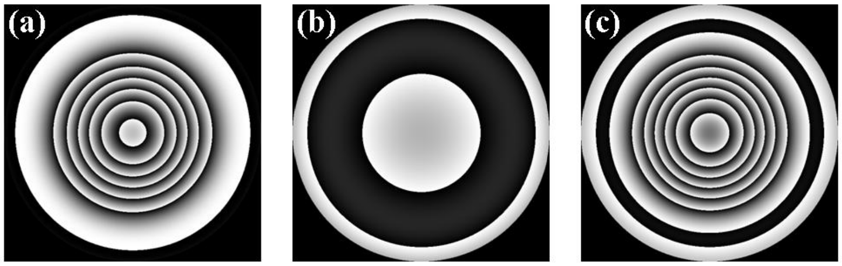

Fig. 2: Representative examples of a SQUBIC phase map (a), a SA pattern (b) and the combination of both (c).

3. Results

Detrimental effects of aberrations are more noticeable as depth location within the sample increases. Fig. 3a shows a MP image acquired at 100 μm inside a piece of cellulose. As expected, the quality of the image is poor due to the effects of the specimen-induced aberrations. This can be over-passed by using a variable SQUBIC mask. There is a particular value of the parameter A providing an MP image with maximum signal (the improvement in signal was 67%, Fig. 3b). However, when this phase pattern is combined with a certain amount of SA, the combination yield an image better than that obtained when using those phase patterns separately (Fig. 3c). For this configuration an enhancement of 98% in signal was found.

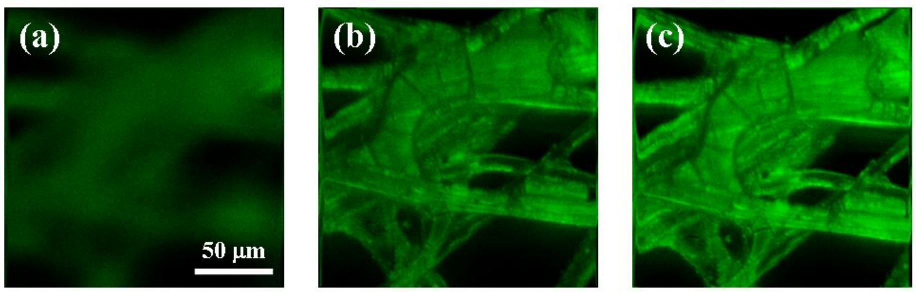

Fig. 3: MP images acquired before (a) and after using the optimum values of SQUBIC (b) and SQUBIC-SA combination (c).

4. Conclusions

We have presented a technique for the reduction of the impact of specimen-induced aberrations in MP imaging. This is based on projecting the combination of SQUBIC and SA phase maps onto the SLM. The dynamic modulation of the laser beam allows image enhancement independently of the sample's thickness. These controlled combinations of phase conditions led to an increase in depth-of-focus, noticeable better than that obtained just using either SQUBIC or SA patterns separately.

5. References

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Acknowledgments

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