Intravital multi-photon imaging through intact highly scattering bone using binary wavefront optimization

Kayvan Forouhesh Tehrani,[1] Peter Kner,[2] Luke J. Mortensen[1,2,*]
[1]*Regenerative Bioscience Center, University of Georgia, Athens, GA 30602, USA;*
[2]*College of Engineering, University of Georgia, Athens, GA 30602, USA*
**luke.mortensen@uga.edu*

Abstract: Diffraction limited imaging of structures in a highly scattering heterogeneous tissue like bone is a non-trivial task. Here we show binary wavefront optimization using a genetic algorithm, for 2-photon imaging of bone endogenous cells.

OCIS codes: (110.1080) Active or adaptive optics; (110.7348) Aberration compensation; (110.0113) Imaging through turbid media; (180.4315) Nonlinear microscopy; (190.4180) Multiphoton processes; (170.3880) Medical and biological imaging

1. Introduction

Multi-photon microscopy achieves deep tissue imaging inside of biological sample by using longer ballistic range of the longer wavelength excitation light [1]. Using near infrared or infrared light provides a longer mean free path compared to visible range light, which expand the achievable depth of penetration. Using longer wavelength and pulsed laser with high peak energy enables optical sectioning at depth such as 500~1000 μm for 2-photon [2] and 3-photon [3] in relatively lower scattering brain tissue, and 150~200 μm depth in high scattering bone tissue [4]. However even though these methods can image deeper, they are not still diffraction-limited, and suffer from scattering aberrations [5]. To fix this issue and restore diffraction limited imaging several wavefront-shaping methods have been proposed. These methods include (but not limited to) using self-healing Bessel beam [6], correction using Zernike model and a deformable mirror (DM) [7], transmission matrix [8], and binary correction [9]. In this research we focus on correction using binary method, because it enables high-speed correction over thousands of segments.

Binary wavefront modulation using a digital micro mirror (DMD) device takes advantage from the fast speed and high number of elements compared to other correction elements. Whereas DMs and spatial light modulators (SLM) can work with refresh rate of up to ~2 kHz, current DMDs can as high as 35 kHz. The importance of high refresh rate becomes significant when we consider the high number of iterations over a large number of segments that a correction algorithm needs to go through, to converge to an optimum position. We previously showed that more than 20,000 segments are required to effectively correct the scattering and achieve a reasonable Strehl ratio for imaging through bone. Therefore use of the right algorithm is important to lower the number of iterations required to converge. Many algorithms including sequential algorithms [10], parallel algorithm [11], genetic algorithm [12], and Hadamard basis [9] have been used to restore the point spread function (PSF) through turbid medium. Genetic algorithm due to its dynamic characteristic has been shown to achieve a solution in a rescannable number of iterations. In this research we use a genetic algorithm to correct the scattering and image endogenous cells inside of the bone marrow cavities.

2. Genetic Algorithm

Genetic Algorithms use an evolutionary approach inspired from biology. These algorithms are based on individuals that are produced from a set of genes that go through evolution, mutate and cross their genes, to improve their capabilities [13]. In the case of binary wavefront optimization, each individual is a wavefront that is made of thousands of segments (genes). In each generation, all the individuals are tested through an intensity metric to find its appropriateness to advance the mission. Each individual is given a weight, and the ones with low weight go through mutation and gene crossing to produce a new generation. This process is repeated until the algorithm converges.

3. Optical Setup

We use a Calamar Cazadero (FLCPA) fiber laser which we frequency double using a BiBo crystal (newlight photonics) to produce a 775nm beam. Power is modulated using a Pockels cell (Conoptics). The beam goes through a pinhole and generates a 4mm beam that is shined on the DMD (TI V-7001 module). The micromirror pitch size of the DMD is 13.7 μm, which - given the 4 mm diameter of the beam – produces an effective area of 66,952

segments. The DMD is conjugated to the back pupil plane of the objective lens, as well as 2 galvanometers for x,y scanning. A 60× Olympus (LUMFLN60×) water immersion objective with NA of 1.1 was used for imaging. Photon multiplier tubes from Hamamatsu (H10770-40) were used for collection of the signal, and their signal was amplified with a transimpedance amplifier (Edmund Optics 59-178). National instruments data acquisitions cards and field programmable gate array module were used for control and synchronization of the system and digitizing of the amplified SHG signal.

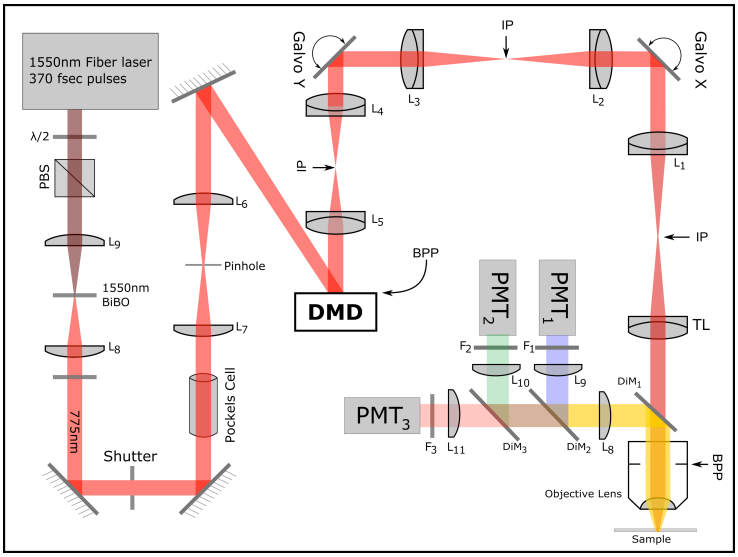

Figure 1 - System diagram of 2-photon binary wavefront optimization microscope. PBS is polarized beam splitter, IP is image plane, BPP is back pupil plane, TL is tube lens, DiM is dichroic mirror, PMT is photon multiplier tube, DMD is digital micro mirror device.

4. Discussion and conclusion

Having an area of about 67k segments enables high level of enhancement, for imaging through 150~200 µm layer of bone. Approximated with $N/2\pi$ [10], where N is the number of segments, beam enhancement can reach a factor of ~10,000. Since we are blocking the parts of the beam that are not contributing to PSF, we are losing some part of the light, which affects the enhancement factor. However the achieved improvement in the PSF will compensate for the loss. Because this method does not depend of the scattering to focus, it can be applied to both high and low scattering tissues.

5. References

1. W. Denk, J. Strickler, and W. Webb, "Two-photon laser scanning fluorescence microscopy," Science **248**, 73-76 (1990).
2. P. Theer, M. T. Hasan, and W. Denk, "Two-photon imaging to a depth of 1000 microm in living brains by use of a Ti:Al2O3 regenerative amplifier," Opt Lett **28**, 1022-1024 (2003).
3. D. Kobat, M. E. Durst, N. Nishimura, A. W. Wong, C. B. Schaffer, and C. Xu, "Deep tissue multiphoton microscopy using longer wavelength excitation," Opt Express **17**, 13354-13364 (2009).
4. L. J. Mortensen, C. Alt, R. Turcotte, M. Masek, T.-M. Liu, D. C. Côté, C. Xu, G. Intini, and C. P. Lin, "Femtosecond laser bone ablation with a high repetition rate fiber laser source," Biomedical Optics Express **6**, 32-42 (2015).
5. K. F. Tehrani, P. Kner, and L. J. Mortensen, "Characterization of wavefront errors in mouse cranial bone using second-harmonic generation," J Biomed Opt **22**, 036012-036012 (2017).
6. G. Thériault, M. Cottet, A. Castonguay, N. McCarthy, and Y. De Koninck, "Extended two-photon microscopy in live samples with Bessel beams: steadier focus, faster volume scans, and simpler stereoscopic imaging," Frontiers in Cellular Neuroscience **8**, 139 (2014).
7. X. Tao, A. Norton, M. Kissel, O. Azucena, and J. Kubby, "Adaptive optical two-photon microscopy using autofluorescent guide stars," Optics Letters **38**, 5075-5078 (2013).
8. M. Kim, W. Choi, Y. Choi, C. Yoon, and W. Choi, "Transmission matrix of a scattering medium and its applications in biophotonics," Optics Express **23**, 12648-12668 (2015).
9. X. Tao, T. Lam, B. Zhu, Q. Li, M. R. Reinig, and J. Kubby, "Three-dimensional focusing through scattering media using conjugate adaptive optics with remote focusing (CAORF)," Optics Express **25**, 10368-10383 (2017).
10. I. M. Vellekoop and A. P. Mosk, "Phase control algorithms for focusing light through turbid media," Optics Communications **281**, 3071-3080 (2008).
11. M. Cui, "Parallel wavefront optimization method for focusing light through random scattering media," Optics Letters **36**, 870-872 (2011).
12. X. Zhang and P. Kner, "Binary wavefront optimization using a genetic algorithm," Journal of Optics **16**, 125704 (2014).
13. K. F. Tehrani, J. Xu, Y. Zhang, P. Shen, and P. Kner, "Adaptive optics stochastic optical reconstruction microscopy (AO-STORM) using a genetic algorithm," Optics Express **23**, 13677-13692 (2015).