

# WHICH SENSORLESS ADAPTIVE OPTICS METHOD IS BEST FOR YOUR MICROSCOPE?

**Qi Hu, Jingyu Wang, Martin Hailstone, Matthew Wincott, Jacopo Antonello,  
Martin J. Booth**

**Department of Engineering Science, University of Oxford, UK**

**E-mail: {qi.hu, martin.booth}@eng.ox.ac.uk**

**KEYWORDS:** Two-photon imaging, sensorless adaptive optics methods, Zernike-based modal method, pupil segmentation, piston-based method

Adaptive optics is an important technique for compensation of aberrations to improve image quality in microscopy. Sensorless adaptive optics (AO) methods are particularly widespread, due to their simple hardware implementation [1]. They work by taking a sequence of images with different applied aberrations in order to infer the necessary aberration correction. The range of sensorless AO schemes that exist have different physical implementations, but can all be fitted into a general mathematical framework that permits comparison between the schemes. One of the major differences among these sensorless methods is that they employ different aberration representations, and hence different modes to modulate and correct phase aberrations. These range from piston correction over many pupil zones [2] [3], through pupil segmentation approaches correcting piston, tip and tilt over individual zones [4] [5], to full pupil modal correction [6]. In order to compare the effectiveness of these methods, we simulated a two-photon microscope imaging a 3D object under random system aberrations; we used the range of sensorless AO methods to correct the same aberration and compare the output image quality after correction. We expect the general trends revealed through studying the two-photon microscope will also be observed in other imaging systems. Results showed that the modal method performs better in general, while small zone methods offer better correction when the initial aberration has low spatial coherence (complex shapes). In the case when noise becomes significant in the imaging system, modal methods are much more robust than small zone methods. Our experimental results on both fluorescence beads and biological samples also support our conclusions drawn from simulation results. These conclusions provide a guideline when selecting a more suitable sensorless AO method to correct a certain type of aberration; they provide us with a deeper understanding of all sensorless methods and also help to develop new sensorless AO schemes.

- [1] M. J. Booth and B. R. Patton, Chapter 2 – “Adaptive optics for fluorescence microscopy,” in *Fluorescence Microscopy*, Boston, Academic Press, 2014, pp. 15-33.
- [2] J. Tang, R. N. Germain and M. Cui, “Superpenetration optical microscopy by iterative multiphoton adaptive compensation technique,” *Proceedings of the National Academy of Sciences*, vol. 109, pp. 8434-8439, 2012.
- [3] L. Kong and M. Cui, “In vivo fluorescence microscopy via iterative multi-photon adaptive compensation technique,” *Opt. Express*, pp. 23786-23794, 2014.
- [4] N. Ji, D. E. Milkie and E. Betzig, “Adaptive optics via pupil segmentation for high-resolution imaging in biological tissues,” *Nature methods*, vol. 7, pp. 141-147, 2009.
- [5] D. E. Milkie, E. Betzig and N. Ji, “Pupil-segmentation-based adaptive optical microscopy with full-pupil illumination,” *Opt. Lett.*, vol. 36, pp. 4206-4208, 2011.
- [6] M. J. Booth, M. A. A. Neil and T. Wilson, “Aberration Correction for Confocal Imaging in Refractive Index Mismatched Media,” *J Microsc*, vol. 192, pp. 90-98, 1998.