

# IMAGE-BASED ABERRATION CORRECTION IN TWO-PHOTON MICROSCOPY

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Adaptive optics systems are aimed at compensating for the wave front aberrations from the optical system and the specimen by use of an adaptive element that introduces a suitable wave front deformation. One approach for optimising this deformation is to use model-based, wavefront sensor-less schemes, in which the aberration correction is indirectly optimised through the application of a short sequence of trial aberrations. Such schemes have been demonstrated in confocal microscopy [1] and more recently in incoherent transmission imaging and structured illumination microscopy [2,3]: by using an appropriate combination of optimisation metric, modal aberration expansion and aberration estimator algorithm, correction can be achieved with a minimal number of measurements.

A critical point in the design of an efficient wave front sensorless adaptive scheme is the appropriate choice of the modes used to describe the aberrations, which allows performing sequential correction of each aberration mode, thereby minimising the total required number of measurements. Here we present a new method for deriving this appropriate set of modes as a function of the optimisation metric and of the image formation process in the imaging system. We show that depending on the systems, this set can be determined either theoretically or experimentally.

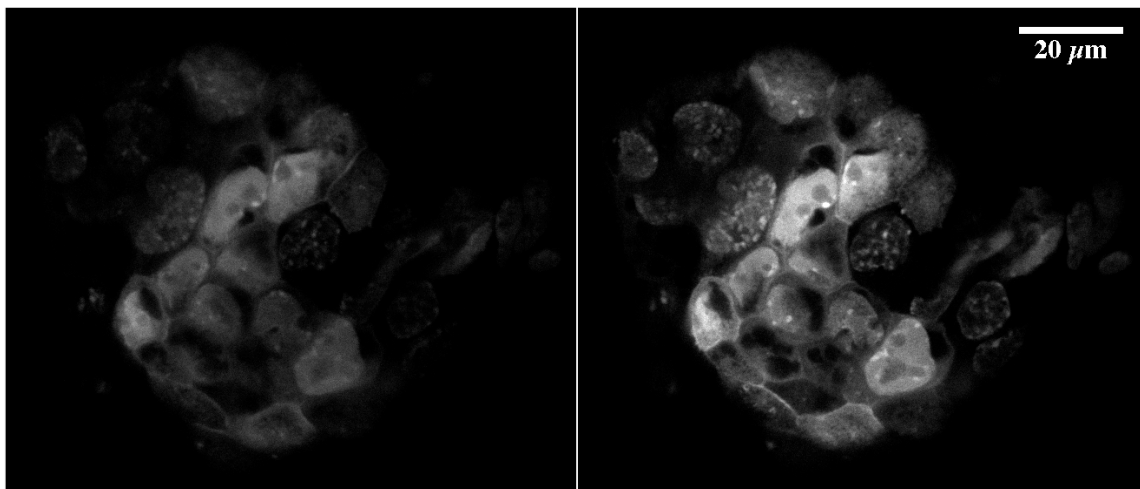


Figure 1: Aberration correction in a two-photon microscope: left – sectioned image of a GFP-stained mouse embryo before correction; right – corresponding image after correction.

Finally, we demonstrate the use of this method for the implementation of wave front sensorless aberration correction in a two-photon microscope. We show that with the obtained correction scheme, the optimal wavefront correction is reached after only one run of the estimator, thereby minimizing the induced photobleaching, and that the contrast of small features is greatly improved after correction. Finally, we demonstrate the use of adaptive two-photon microscopy for imaging various biological samples.

## REFERENCES

- [1] M. J. Booth *et al.*, “Adaptive aberration correction in a confocal microscope”, *Proc. Natl. Acad. Sci. USA* **99**, 5788-5792 (2002)
- [2] D. Débarre *et al.*, “Image based adaptive optics through optimisation of low spatial frequencies”, *Optics Express* **15**, 8176-8190 (2007)
- [3] D. Débarre *et al.*, “Adaptive optics for structured illumination microscopy”, *Optics Express* **16**, 9290-9305 (2008)