# ADVANCED ADAPTIVE OPTICS METHODS FOR 4PI SINGLE MOLECULE SUPERRESOLUTION MICROSCOPY

**Jingyu Wang[1], Jacopo Antonello[1], Richard M. Parton[2], Chao He[1], Ian M. Dobbie[2], Ilan Davis[2], Martin J Booth[1]**

**[1]Department of Engineering Science, University of Oxford, UK**

**[2]Department of Biochemistry, University of Oxford, Oxford, United Kingdom**
**Email: jingyu.wang@eng.ox.ac.uk**

**KEYWORDS**: Adaptive optics, 4Pi microscope, super-resolution, single molecule localization, three-dimensional imaging, wavefront sensing

We present a unique adaptive optics method on a 4Pi single molecule switching (SMS) microscope for ultra-high spatial resolution single molecule imaging in cells.

Single molecule switching (SMS) microscopy can provide nanometre spatial resolution for biological studies in fixed cells; however, the axial resolution of standard SMS microscopes is inherently inferior to the transverse resolution. A classic way of achieving isotropic 3D resolution in nanometres is by using two opposing objective lenses for coherent detection. A SMS microscope in this so-called 4Pi configuration enables ultra-high axial resolution with an improved signal collection efficiency. However, due to the nature of 4Pi imaging, even a moderate sample thickness can introduce aberrations that affect the imaging performance of the system. More importantly, the aberrations experienced by the two imaging paths of the 4Pi configuration are different and will vary differently as the imaging position moves in 3D. For these reasons, the actual imaging resolution in cellular samples deteriorate significantly from when imaging plane moves deep inside the cells. This limits the axial imaging range and makes imaging large cells or tissues with uncompromised resolution impossible unless the sample induced aberrations are compensated using adaptive optics (AO) techniques [1][2].

The nature of aberrations in a 4Pi configuration and its effect on the system performance have been described together with a new mathematical representation of these aberrations - 4pi aberration modes [3]. Based on this knowledge, we experimentally demonstrate aberration correction methods using a wavefront-sensorless AO approach specifically designed for 4Pi aberration modes. A pair of deformable mirrors is used, one for each high numerical-aperture objective, that work together to control a set of 4Pi aberration modes. We estimate the aberrations based on unique interferometric imaging metrics and compare the performance of this method with AO correction through the separate objective paths [1] for three-dimensional cell imaging. These methods will extend the imaging depth limits of advanced 4Pi nanoscopic imaging, which will be extremely valuable in studying whole cells, large cells and tissues.

[1] Huang, F., Sirinakis, G., et al., 2016. “Ultra-high resolution 3D imaging of whole cells”. *Cell*, *166*(4), pp.1028-1040 (2016).
[2] Wang, J., Allgeyer, E.S., Sirinakis, G. et al. Implementation of a 4Pi-SMS super-resolution microscope. Nat Protoc (2020). https://doi.org/10.1038/s41596-020-00428-7
[3] Xiang Hao, Jacopo Antonello, Edward S. Allgeyer, Joerg Bewersdorf, and Martin J. Booth, "Aberrations in 4Pi Microscopy," *Opt. Express* 25, 14049-14058 (2017)